Phenolic constituents from twigs of *Aleurites fordii* and their biological activities

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**Full Research Paper**

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**Abstract**

Three new neolignan glycosides (1–3), a new phenolic glycoside (15), and a new cyanoglycoside (16) were isolated and characterized from the twigs of *Aleurites fordii* together with 14 known analogues (4–14 and 17–19). The structural elucidation of the new compounds was performed through the analysis of their NMR, HRMS, and ECD spectra and by chemical methods. All isolated compounds were tested for their antineuroinflammatory and neuroprotective activities.

**Introduction**

*Aleurites fordii* Hemsl. (= *Vernicia fordii* Hemsl., Euphorbiaceae), known as tung oil tree, is widely distributed throughout Northeast Asia [1]. The fruits, leaves, and roots of this plant have been used as a Korean traditional medicine for treating sore throat, respiratory illness, constipation, and diuresis [2,3]. Phytochemical investigations of *A. fordii* reported coumarins, diterpenoid esters, triterpenoids, and tannins [4-7]. Some phorbol diterpenoids isolated from *A. fordii* have shown Epstein–Barr virus activation effects and an enhancement of HTLV-I-induced colony formation of lymphocytes [8]. As an ongoing search for bioactive secondary metabolites from Korean medicinal sources, we investigated the methanolic extract of the twigs of *A. fordii* which resulted in the isolation and characterization of 14 lignan derivatives including three new neolignan glycosides (1–3), four phenolic glycosides including a new compound (15), and a new cyanoglycoside (16) from the organic extracts. The structures of the new compounds were established by NMR analysis (1H and 13C NMR, COSY, HSQC, HMBC, and NOESY), HRMS, and chemical methods. The isolated compounds 1–19 were evaluated for their...
antineuroinflammatory and neuroprotective activities. In this paper, we report the isolation and structural elucidation of these phytochemicals and their biological activity.

Results and Discussion
The MeOH extract of *A. fordii* twigs was subjected to liquid–liquid solvent partitioning to yield *n*-hexane, CHCl₃, EtOAc, and *n*-BuOH-soluble fractions. Repeated column chromatographic purification of the CHCl₃, EtOAc, and *n*-BuOH-soluble fractions afforded three new neolignan glycosides (1–3), a new phenolic glycoside (15), a new cyanoglucoside (16), and 14 known compounds (4–14 and 17–19) (Figure 1).

Compound 1 was obtained as a colorless gum. The molecular formula was determined to be C₂₅H₃₂O₁₁ from the [M + Na]⁺ molecular ion peak in the positive mode HRFABMS. The

![Figure 1: Chemical structures of compounds 1–19.](image-url)
$^1$H NMR data (Table 1) of compound 1 displayed characteristic resonances for a 1,3,4-trisubstituted benzene ring [$\delta_H 7.00$ (d, $J = 1.9$ Hz, H-2), 6.87 (dd, $J = 8.1, 1.9$ Hz, H-6), and 6.78 (d, $J = 8.1$ Hz, H-5)], a 1,3,4,5-tetrasubstituted benzene ring [$\delta_H 6.62$ (brs, H-2') and 6.65 (brs, H-6')], an oxygenated methine [$\delta_H 5.51$ (d, $J = 6.1$ Hz, H-7)], an anomeric proton of a sugar [$\delta_H 4.27$ (d, $J = 7.8$ Hz, H-1')], and a methoxy group [$\delta_H 3.84$ (s, 3-OCH$_3$)]. The $^{13}$C NMR data (Table 1) showed 25 peaks including 12 aromatic carbons [$\delta_C$ 147.6 (C-3), 145.9 (C-4), 145.1 (C-4'), 140.4 (C-3'), 135.2 (C-1'), 133.7 (C-1), 128.3 (C-5), 118.2 (C-6), 115.8 (C-2'), 115.3 (C-6'), 114.6 (C-5), and 109.1 (C-2')] and six glucose carbons [$\delta_C$ 103.0 (C-5'), 115.3 (C-6'), 114.6 (C-5'), 118.2 (C-6'), 115.8 (C-2'), 115.3 (C-6')]. The spectroscopic data of compound 1 suggested that it is a typical dihydrobenzofuran neo lignan glycoside [9-11]. The data for compound 1 were similar to those of glochidiobiolide isolated from Glochidion obovatum [12], except for the presence of a hydroxy group instead of the methoxy group at C-3' in 1. The two-dimensional structure of 1 was elucidated via analysis of COSY, HSQC, and HMBC spectroscopic data (Figure 2). The locations of the glucose unit and the methoxy group were confirmed from the observed HMBC correlations of H-1'/C-9' and 3-OCH$_3$/C-3, respectively (Figure 2). Acid hydrolysis of 1 was conducted to analyze the aglycone and sugar moiety. The structure of the aglycone (1a) was confirmed as demethyldihydrodehydrodiconiferyl alcohol based on the comparison of $^1$H NMR and MS data [13]. The relatively large coupling constant of the anomeric proton

Table 1: $^1$H and $^{13}$C NMR spectroscopic data of compounds 1-3 in CD$_3$OD.

| Pos. | $^1$H ($\delta_C$) | $^1$H ($\delta_H$ in Hz) | $^1$H ($\delta_C$) | $^1$H ($\delta_H$ in Hz) | $^1$H ($\delta_C$) | $^1$H ($\delta_H$ in Hz) |
|------|------------------|--------------------------|------------------|--------------------------|------------------|--------------------------|
| 1    | 133.7            | 134.3                    | 133.7            | 134.3                    | 133.7            | 134.3                    |
| 2    | 109.1            | 112.3                    | 7.06, d (2.0)    | 112.2                    | 6.58, d (2.3)    |
| 3    | 147.6            | 152                      | 146.9            |                          |
| 4    | 145.9            | 146.6                    | 143.9            |                          |
| 5    | 114.6            | 119.4                    | 7.12, d (8.0)    | 114.2                    | 6.57 d (8.0)     |
| 6    | 118.2            | 120.1                    | 6.92, dd (8.0, 2.0) | 121.2                    | 6.49 dd (8.0, 2.3) |
| 7    | 87.3             | 88.3                     | 5.85, d (8.8)    | 37.8                     | 2.99 dd (13.8, 5.5)|
|      |                  |                          |                  |                          | 2.72 dd (13.8, 9.6) |
| 8    | 54.3             | 50.2                     | 3.69, m          | 41.3                     | 3.98, m           |
| 9    | 63.7             | 63.6                     | 3.31, m          | 65.6                     | 3.77 dd (10.7, 6.2)|
|      |                  |                          |                  |                          | 3.68 dd (10.7, 7.3) |
| 1'   | 135.2            | 137.1                    |                  |                          | 138.9            |
| 2'   | 115.8            | 114.3                    | 6.76, br s       | 110.3                    | 6.73, s           |
| 3'   | 140.4            | 145.3                    |                  |                          | 151.6            |
| 4'   | 145.1            | 147.5                    |                  |                          | 142.2            |
| 5'   | 128.3            | 131.9                    |                  |                          | 137.1            |
| 6'   | 115.3            | 119.2                    | 6.85, br s       | 118.9                    | 6.73, s           |
| 7'   | 31.2             | 33.1                     | 2.65, t (7.5)    | 31.7                     | 2.66, m          |
| 8'   | 31.5             | 35.9                     | 1.85, m          | 34.1                     | 1.84, tt (13.0, 6.5) |
| 9'   | 68.5             | 62.4                     | 3.59, t (6.5)    | 60.8                     | 3.59, td (6.5, 1.9) |
|      |                  |                          | 3.56, m          |                          |                  |
| 1''  | 103.0            | 101.6                    | 5.37, d (1.8)    | 104.2                    | 4.63 d (7.6)     |
| 2''  | 73.7             | 72.2                     | 4.08, m          | 74.5                     | 3.47, m          |
| 3''  | 76.7             | 72.4                     | 3.89, m          | 76.4                     | 3.42, m          |
| 4''  | 70.2             | 74                       | 3.47, m          | 69.8                     | 3.39, m          |
| 5''  | 76.5             | 71                       | 3.83, m          | 76.6                     | 3.14 ddd (9.2, 5.2, 2.3) |
| 6''  | 61.3             | 18.1                     | 1.24, d (6.2)    | 61.0                     | 3.08 ddd (11.0, 8.7) |
|      |                  |                          |                  |                          | 3.70, overlap    |
| 3-OCH$_3$ | 54.9          | 56.9                     | 3.89, s          | 54.8                     | 3.71, s          |
| 3'-OCH$_3$ | 56.6          | 54.9                     | 3.83, s          | 54.9                     | 3.82, s          |

$^a$Measured at 700 ($\delta_C$) and 175 ($\delta_H$) MHz. $^b$Measured at 500 ($\delta_C$) and 125 ($\delta_H$) MHz.
(7.8 Hz) confirmed that the glucose is combined as β-form [14]. n-Glucose was identified by co-TLC with a standard sample [CHCl₃/MeOH/H₂O 2:1:0.1, Rf = 0.3] and GC–MS analysis [15]. The relative configuration at C-7/C-8 of I was established as trans through the relatively small coupling constant (6.1 Hz) [10,16]. The analysis of the ECD spectrum of I determined the absolute configuration of 1 to be 7S and 8R (positive Cotton effects (CEs) at 292 and 248 nm, and a negative CE at 221 nm; see Supporting Information File 1, Figure S7) [10]. Thus, the structure of compound 1 was elucidated as (7S,8R)-3′-demethyldihydrodehydrodiconiferyl alcohol 9′-O-β-d-glucopyranoside, and was named as aleuritiside A.

The molecular formula of compound 2, isolated as a colorless gum, was confirmed to be C₂₆H₃₄O₁₀ from the positive ion mode HRESIMS data. The ¹H and ¹³C NMR spectra of 2 were very close to that of icariside E₄ [17] with significant differences in the chemical shifts of C-1, C-8, C-9, C-5′, and C-6′ [2: δC 134.3, 50.2, 63.6, 131.9, and 119.2; 5: δC 138.9, 55.7, 65.1, 129.7, and 118.0, respectively], indicating that compound 2 could be a stereoisomer of 5 at C-7 and C-8. The inspection of the COSY, HSQC, and HMBC spectra confirmed the planar structure of 2. The HMBC correlation of H-1′″ to C-4 indicated that the rhamnose unit was linked to the oxygen at C-4 and the characteristic J value of the anomeric proton (1.5 Hz) confirmed the rhamnose as α-form (Figure 2) [10]. Acid hydrolysis of compound 2 afforded the aglycone, dihydrodehydrodiconiferyl alcohol (2a) [18], and l-rhamnose ([α]D²⁵ +9.0), which was identified in an identical manner to that of compound 1. The relatively large coupling constant (8.8 Hz) between H-7 and H-8 in 2, as opposed to the relatively small coupling constant (6.1 Hz) between H-7 and H-8 in I, verified that H-7 and H-8 are cis-oriented [10,16], which was supported by the NOESY correlations of H-7/H-8, H-2/H-9, and H-6/H-9 (Figure 2). The ECD spectrum of 2 showed negative CEs at 276 nm and 229 nm and a positive CE at 248 nm, indicating the absolute configuration of C-7 and C-8 as R (Figure S15 in Supporting Information File 1) [19]. Therefore, the structure of compound 2 was determined to be (7R,8R)-dihydrodehydrodiconiferyl alcohol 4-O-α-L-rhamnopyranoside and was named as aleuritiside B.

Compound 3 was obtained as a colorless gum after purification with a molecular formula of C₂₆H₃₆O₁₁ as deduced from the positive molecular ion peak [M + Na]⁺ at m/z 547.2155 (calcd for C₂₆H₃₆O₁₁Na, 547.2155) in the HRESIMS. Analysis of the ¹H and ¹³C NMR data (Table 1) showed a 1,3,4-trisubstituted benzene ring [δH 6.58 (d, J = 2.3 Hz, H-2), 6.57 (d, J = 8.0 Hz, H-3), 6.56 (d, J = 8.0 Hz, H-4), 6.54 (d, J = 2.3 Hz, H-5)].
Table 2: $^1$H and $^{13}$C NMR spectral data of 15 and 16 in CD$_3$OD.

| pos. | $\delta$C | $\delta$H (J in Hz) | $\delta$C | $\delta$H (J in Hz) |
|------|-----------|----------------------|-----------|----------------------|
| 1    | 134.6     | 7.23, s              | 121.3     |                     |
| 2    | 107.5     | 7.23, s              | 118.2     |                     |
| 3    | 154.4     | 7.23, s              | 133.2     | 5.97, br s          |
| 4    | 140.7     | 7.23, s              | 69.3      | 4.34, dd (13.7, 1.5)|
| 5    | 154.4     | 7.23, s              | 5.95, br s | 4.22, dd (13.7, 1.5)|
| 6    | 107.5     | 7.23, s              | 5.00, d (7.7) | 4.70, d (8.0)    |
| 7    | 200.0     | 5.00, d (7.7)        | 102.1     | 3.70, m             |
| 8    | 42.1      | 3.76, dd (12.0, 2.0) | 75.8      | 4.99, dd (9.5, 8.0) |
| 9    | 58.8      | 3.76, dd (12.0, 2.0) | 75.2      | 3.75, m             |
| 1'   | 104.6     | 3.76, dd (12.0, 2.0) | 78.6      | 3.72, m             |
| 2'   | 75.8      | 3.76, dd (12.0, 2.0) | 71.5      | 3.67, m             |
| 3'   | 78.6      | 3.76, dd (12.0, 2.0) | 71.8      | 3.67, m             |
| 4'   | 78.1      | 3.76, dd (12.0, 2.0) | 76.2      | 4.57, dd (12.0, 2.0) |
| 5'   | 62.7      | 3.76, dd (12.0, 2.0) | 64.5      | 4.46, dd (12.0, 5.0) |
| 6'   | 75.9      | 3.76, dd (12.0, 2.0) | 77.2      |                     |
| 7'   | 3.76, dd (12.0, 5.0) | 3.66, dd (12.0, 5.0) | 4.46, dd (12.0, 5.0) | 3.66, dd (12.0, 5.0) |
| 3.5-OCH$_3$ | 57.3      | 3.81, s              | 121.4     |                     |

a Measured at 700 ($\delta$C) and 175 ($\delta$H) MHz. b Measured at 500 ($\delta$H) and 125 ($\delta$C) MHz.
exhibited signals for a 1,3,4,5-tetrasubstituted aromatic ring [δ_H 7.23 (s, 2H, H-2 and H-6)], two methoxy groups [δ_H 3.81 (s, 6H, 3,5-OCH_3)], an anomeric proton [δ_H 5.00 (d, J = 7.7 Hz, 1H, H-1′)], and two methylenes [δ_H 3.23 (t, J = 6.0 Hz, 2H, H-8) and 3.96 (t, J = 6.0 Hz, 2H, H-9)]. The 13C NMR spectrum of compound 15 (Table 2) revealed 14 peaks for 17 carbons including a ketone carbon (δ_C 200.0), a 1,3,4,5-tetrasubstituted aromatic ring [δ_C 154.4 (×2), 140.7, 134.6, and 107.5 (×2)], two methoxy groups [δ_C 57.3 (×2)], and a glucose unit (δ_C 104.6, 78.6, 78.1, 75.8, 71.5, and 62.7). The location of the glucose unit was determined to be at C-4 by analysis of the HMBC data showing a correlation from H-1 to C-4 (Figure 2). The HMBC cross-peaks were in good agreement with literature data reported from Beilstein J. Org. Chem. 17, 2329–2339. Consequently, the structure of 16 was determined to be codiacyanoglusyl-2′,6′-O-digallate, named aleucyanoglucoside.

The other known compounds were identified as 7R,8S-dihydrodehydrodiconiferyl alcohol 4-O-β-D-glucopyranoside (4) [26], icariside E5 (4)[17], isomassonianoside B (6) [27], sakuraresinol (7) [28], selaginellol 4′-O-β-D-glucopyranoside (8) [21], 7R,8R-4,7,9,9′-tetrahydroxy-3,3′-dimethoxy-8-O-4′-neolignan-7-O-β-D-glucopyranoside (9) [29], 7R,8R-4,9,9′-trihydroxy-3,3′-dimethoxy-8-O-4′-neolignan (10) [30], 7,8,8′,9,9′-trihydroxy-3,3′-dimethoxy-8-O-4′-neolignan (11) [30], 7R,8S,4,7,9,9′-tetrahydroxy-3,3′-dimethoxy-8-O-4′-neolignan-7-O-β-D-glucopyranoside (12) [29], 7,8R,4-7,9-trihydroxy-3,3′-dimethoxy-8-O-4′-neolignan-9′-O-β-D-glucopyranoside (13) [31], buddalenol A (14) [32], aurilin (17) [33], fraxinol (18) [34], and 5,6,7-trimethoxy coumarin (19) [34] based on the comparison of their spectroscopic data and specific rotation with the reported data.

Compounds 1–19 were tested for their effects on nitric oxide (NO) production levels in lipopolysaccharide (LPS)-stimulated murine microglial BV-2 cells to evaluate for antineuroinflammatory activities (Table 3). Compound 14 showed relative inhibitory effects on NO production with an IC_{50} value of 20.9 µM which was stronger than the positive control (L-NMMA, IC_{50} 28.8 µM). Compounds 11, 17, and 19 also displayed moderate activity (IC_{50} 35.5–37.1 µM), whereas compounds 2, 4, 10, 12, and 13 exhibited only weak effects (IC_{50} 42.1–55.0 µM). Interestingly, compounds 2 and 5 have the same planar structures with only differing in the C-7 stereochemistry, but they showed quite different inhibition effects on NO production (IC_{50} 55.0 µM, 2; IC_{50} > 500 µM, 5). The MTT cell viability test suggested that all the compounds had no cytotoxic effect on BV-2 cell survival at a concentration of 20 µM.

Compounds 1–19 were also tested for their neuroprotection activity by measuring the secretion of NGF from C6 cells into the medium (Table 4). Compounds 8 and 16 stimulated NGF release, exhibiting stimulation levels of 134.2 ± 8.1% and 134.6 ± 5.9%, respectively. Although compounds 3 and 8 have similar structures without or with a methoxy group at C-5, respectively, only compound 8 showed a significant activity (96.2 ± 1.1% for 3). The other compounds exhibited moderate or no NGF secretion effect.

The cytotoxicity of compounds 1–19 was also evaluated against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and

Bellstein J. Org. Chem. 2021, 17, 2329–2339.
Table 3: Effects of isolated compounds on NO production in LPS-activated BV-2 cells.

| comp. | IC$_{50}$ (µM)$^a$ | cell viability$^b$% | comp. | IC$_{50}$ (µM)$^a$ | cell viability$^b$% |
|-------|-------------------|---------------------|-------|-------------------|---------------------|
| 1     | >500              | 87.5 ± 5.1          | 11    | 37.1              | 89.97 ± 3.2         |
| 2     | 55.0              | 90.3 ± 3.1          | 12    | 47.6              | 90.1 ± 3.7          |
| 3     | 109.8             | 93.1 ± 3.5          | 13    | 42.1              | 87.9 ± 4.2          |
| 4     | 48.6              | 86.9 ± 6.5          | 14    | 20.9              | 105.2 ± 1.5         |
| 5     | >500              | 111.9 ± 4.3         | 15    | 93.7              | 93.3 ± 7.4          |
| 6     | 74.6              | 86.2 ± 6.2          | 16    | >500              | 95.8 ± 4.2          |
| 7     | 278.8             | 90.2 ± 4.1          | 17    | 35.5              | 101.8 ± 4.2         |
| 8     | 126.6             | 87.9 ± 5.1          | 18    | 117.0             | 93.6 ± 4.4          |
| 9     | 321.7             | 87.9 ± 7.6          | 19    | 36.7              | 99.7 ± 2.9          |
| 10    | 42.9              | 86.3 ± 4.4          |       | L-NMMA$^c$        | 28.8 ± 3.6          |

$^a$The IC$_{50}$ value of each compound was defined as the concentration (µM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells.

$^b$The cell viability after treatment with 20 µM of each compound was measured using the MTT assay and is expressed as a percentage (%). Results are the means of three independent experiments, and data are expressed as the means ± SD.

$^c$Positive control substance.

Table 4: Effects of isolated compounds on NGF secretion in C6 cells.

| comp. | NGF secretion$^a$ (%) | cell viability$^b$ (%) | comp. | NGF secretion$^a$ (%) | cell viability$^b$ (%) |
|-------|-----------------------|-----------------------|-------|-----------------------|-----------------------|
| 1     | 117.4 ± 2.8           | 92.7 ± 1.3            | 11    | 102.1 ± 5.4           | 92.3 ± 0.3            |
| 2     | 101.3 ± 7.6           | 90.5 ± 0.1            | 12    | 110.3 ± 0.8           | 94.0 ± 1.9            |
| 3     | 96.2 ± 1.1            | 91.0 ± 0.8            | 13    | 104.5 ± 3.2           | 92.9 ± 0.2            |
| 4     | 99.1 ± 1.0            | 93.9 ± 1.1            | 14    | 12.0 ± 0.4            | 99.6 ± 3.1            |
| 5     | 102.5 ± 8.0           | 91.1 ± 2.0            | 15    | 111.3 ± 7.6           | 98.0 ± 4.1            |
| 6     | 113.9 ± 0.9           | 93.0 ± 0.5            | 16    | 134.6 ± 5.9           | 88.8 ± 3.9            |
| 7     | 101.2 ± 5.8           | 97.9 ± 0.3            | 17    | 102.1 ± 2.5           | 98.7 ± 2.7            |
| 8     | 134.2 ± 8.1           | 94.5 ± 3.9            | 18    | 99.2 ± 2.7            | 97.9 ± 4.3            |
| 9     | 121.0 ± 0.6           | 94.4 ± 1.8            | 19    | 104.1 ± 4.6           | 98.7 ± 3.7            |
| 10    | 109.0 ± 5.3           | 92.9 ± 4.1            |       | 6-shogaol$^c$        | 143.9 ± 12.5          |

$^a$C6 cells were treated with 20 µM of each test compound. After 24 h, the content of NGF secreted in the C6-conditioned medium was measured by ELISA. The level of secreted NGF is expressed as the percentage of the untreated control (set as 100%).

$^b$Cell viability after treatment with 20 µM of each compound was determined by an MTT assay and is expressed as a percentage (%). Results are the means of three independent experiments, and the data are expressed as means ± SD.

$^c$Positive control substance.

Conclusion

Isolation of phytochemical constituents from the twigs of *A. fordii* led to the discovery of three new neolignan glycosides 1–3, a new phenolic glycoside 15, and a new cyanoglycoside 16 along with 14 known compounds 4–14 and 17–19. The structural characterization of the new compounds was conducted based on the analysis of their spectroscopic and spectrometric data, and chemical methods. All isolated compounds were tested for their antineuroinflammatory and neuroprotective activities. Compound 14 showed inhibition effects on NO production and the stereoisomers 2 and 5 demonstrated the difference in activity according to the configuration. Compounds 8 and 16 exhibited neuroprotection effects. Thus, this study indicates that the active phenolic compounds from *A. fordii* would be potential candidates for drug discovery associated with antineurodegenerative diseases.

Experimental

**General experimental procedures.** Optical rotations were measured on a JASCO P-2000 polarimeter. IR spectra were acquired with a JASCO FT/IR-4600 spectrometer. UV spectra were obtained on a Shimadzu UV-1601 UV–visible spectrophotometer. NMR spectra were recorded on a Bruker AVANCE III 700 NMR spectrometer operating at 700 MHz ($^1$H) and 175 MHz ($^{13}$C) with chemical shifts given in ppm (δ).
and a Varian UNITY INOVA 500 NMR spectrometer (Varian Palo Alto, CA, USA) operating at 500 MHz (1H) and 125 MHz (13C). HRESIMS spectra were obtained on a Waters SYNAPT G2 mass spectrometer and semipreparative HPLC was conducted using a Gilson 306 pump with a Shodex refractive index detector and a Phenomenex Luna 10 μm column (250 × 10 mm). Silica gel 60 (Merck, Darmstadt, 70–230 mesh, and 230–400 mesh) and RP-C18 silica gel (Merck, 230–400 mesh) were used for column chromatography. Low-pressure liquid chromatography was performed over Merck LiChroprep Lobar-A Si gel 60 (240 × 10 mm) with an FMI QSY-0 pump (ISCO). Merck precoated silica gel F254 plates and RP-18 F254a plates were used for TLC. Spots were detected on TLC under UV light or by heating after spraying the samples with anisaldehyde-sulfuric acid.

Plant material. Twigs of *A. fordii* were collected in Chungbuk Goesan, Korea in August 2012 and the plant was identified by Dr. Kang Ro Lee, Professor at Sungkyunkwan University. A voucher specimen (SKKU-NPL 1212) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Republic of Korea.

Extraction and isolation. Twigs of *A. fordii* (7.0 kg) were extracted three times with 80% aqueous MeOH (each 10 L × 1 day) under reflux and filtered. The filtrate was evaporated under vacuum to obtain a crude MeOH extract (325 g), which was suspended in distilled water and successively partitioned with *n*-hexane, CHCl3, EtOAc, and *n*-BuOH, to yield 15 g, 15 g, 9 g, and 23 g of each residue, respectively. The CHCl3-soluble layer (15.0 g) was separated by Sephadex LH-20 chromatography (80% MeOH) to yield six fractions (C1–C6). Fraction C1 (20 mg) was subjected to RP-C18 silica gel chromatography, eluting with gradient solvent system (30 → 100% MeOH) to yield nine subfractions (E1–E9). Fraction E7 (0.4 g) was subjected to RP-C18 silica gel chromatography, eluting with gradient solvent system (30 → 100% MeOH) to yield nine subfractions (E7A–E7I). Fractions E7A (31 mg), E7B (98 mg), E7C (30 mg), and E7D (37 mg) were purified by semipreparative HPLC (15% MeOH and 25–30% MeOH) to yield compounds 1 (3 mg), 5 (6 mg), and 15 (3 mg). Fraction E8 (0.9 g) was subjected to RP-C18 silica gel chromatography, eluting with gradient solvent system (30 → 100% MeOH) to yield six subfractions (E8A–E8F). Compounds 4 (4 mg), 6 (3 mg), and 16 (4 mg) were obtained by purification of fractions E8B (54 mg) and E8C (120 mg) using semipreparative HPLC (40% MeOH).

The *n*-BuOH-soluble layer (23.0 g) was chromatographed on a Diaion HP-20 column, eluting with an isocratic solvent system of 100% H2O and 100% MeOH, yielding H2O- and MeOH-soluble fractions. The MeOH fraction was subjected to separation on a silica gel column (CHCl3/MeOH/H2O 6:1:0.1 → 1:1:0.1) to afford five fractions (BM1–BM5). Fraction BM3 (2.5 g) was fractionated over an RP-C18 silica gel column, eluting with gradient solvent system (25 → 100% MeOH) to give nine subfractions (BM3A–BM3I). Subfraction BM3D (61 mg) was purified by semipreparative HPLC (23% MeOH) to acquire compound 13 (3 mg). Compounds 3 (8 mg) and 8 (8 mg) were isolated upon purification of subfraction BM3E (120 mg) using semipreparative HPLC (17% MeOH).

Fraction BM4 (1.0 g) was subjected to passage over an RP-C18 silica gel column, eluting with gradient solvent system (15 → 100% MeOH) to acquire 17 subfractions (BM4A–BM4Q). Compounds 1 (5 mg) and 12 (9 mg) were obtained by purification of fraction BM4M (65 mg) using semipreparative HPLC (15% MeOH). Fraction BM4N (58 mg) was purified by semipreparative HPLC (30% MeOH) to yield compound 9 (10 mg).

Aleuritiside A (1). Colorless gum; [α]D25 25° = –12.1 (c 0.05, MeOH); IR (KBr) νmax: 3360, 2943, 2830, 1448, 1033 cm–1; UV (MeOH) λmax, nm (log ε): 282 (1.40), 228 (3.61); ECD (MeOH) λmax, nm (Δε): 292 (5.3), 248 (3.3), 221 (2.1); 1H and 13C NMR data, see Table 1; positive HRMS–FAB (m/z): [M + Na]+ calcd for C25H32O11Na, 531.1837; found, 531.1844.

Aleuritiside B (2). Colorless gum; [α]D25 25° = –15.4 (c 0.05, MeOH); IR (KBr) νmax: 3355, 2945, 2832, 1453, 1033 cm–1; UV (MeOH) λmax, nm (log ε): 283 (1.31), 230 (3.53); ECD...
subsequently passed through an ion exchange column (Dowex® (1 mL) at room temperature for 4 h. The reaction mixture was (1.0 mg) was hydrolyzed with 0.1 N KOH

Alkaline hydrolysis of 16.

Authentic samples hydrolysate with standard silylated samples, giving a single peak at 9.730 and 9.712 min, respectively. Authentic samples showed a single peak at 9.730 and 9.708 min, respectively.

Sample solutions were mixed with deuterium oxide and 1-trimethylsilylimidazole (0.1 mL, Sigma-Aldrich, St. Louis, MO, USA). The mixture was refluxed with 1 mL of 1 N HCl for 1 h at 90 °C. The hydrolysate was dissolved in anhydrous pyridine (0.5 mL) followed by adding of

In a manner similar as described in [35], compounds 1–3 and 15 (each 1.0–2.0 mg) were refluxed with 1 mL of 1 N HCl for 1 h at 90 °C. The hydrolysate was extracted with EtOAc and the aqueous fraction was partitioned between EtOAc/H2O (1:2) using deionized water to remove KOH. A portion of the reaction product was partitioned between EtOAc/H2O (each 1.0 mL) and the aglycone 16a was acquired from the EtOAc-soluble phase.

Measurement of nitric oxide production and cell viability. In a manner similar as described in [37], BV2 cells were used to test the inhibitory effect of the isolated compounds on LPS-stimulated NO production [38,39]. The BV2 cells seeded on a 96-well plate (4 × 104 cells/well) were treated with and without various concentrations of the test compounds. The treated cells were stimulated with LPS (100 ng/mL) and incubated for 24 h. The level of nitrite (NO2, a soluble oxidation product of NO) in the culture medium was measured using the Griess reagent (0.1% N-1-naphthylethylenediamine dihydrochloride and 1% sulphanilamide in 5% phosphoric acid). The supernatant (50 μL) in each well was harvested and mixed with an equal volume of Griess reagent. After 10 min, the absorbance was measured at 570 nm with a microplate reader (Emax, Molecular Devices, Sunnyvale, CA, USA). Graded sodium nitrite solution was used as a standard to gauge NO2 concentration. Cell viability was assessed by the MTT assay.

Measurement of NGF secretion and cell viability assays. C6 glioma cells (Korean Cell Line Bank, Seoul, Republic of Korea) were used to measure the release of nerve growth factor (NGF) into the culture medium. The C6 cells were seeded onto 24-well plates at a density of 1 × 105 cells/well. After 24 h, the cells were treated with serum-free DMEM and incubated with different concentrations of the test compounds for an additional 24 h. The NGF levels were evaluated in the medium supernatant using an ELISA development kit. Cell viability was measured using the MTT assay and the results were expressed as a percentage of the control group (untreated cells).

Cytotoxicity assessment. The SRB assay was performed to evaluate cytotoxicity of all the isolated compounds against four cultured human cancer cell lines. The cell lines (National Cancer Institute, Bethesda, MD, USA) were used A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). Each cell line was inoculated over standard 96-well flat-bottom microplates and incubated for 24 h at 37 °C in condition of a humidified atmosphere of 5% CO2. The attached cells were incubated with various concentrations of the test compounds and the cells were cultured for 48 h. Then, the culture medium was removed from each well and the cells were fixed with 10% cold trichloroacetic acid at 4 °C for 1 h. After the supernatant was discarded and the plates were washed with tap water, the cells were stained with 0.4% SRB solution and incubated for 30 min at room temperature. The stained cells were
washed to remove the unbound dye and subsequently solubilized with 10 mM unbuffered Tris base solution (pH 10.5). The absorbance was measured spectrophotometrically at 520 nm with a microtiter plate reader. Etoposide (Sigma Chemical Co., ≥98%) was used as a positive control.

Supporting Information

Supporting Information File 1
Copies of NMR spectra including 1D and 2D NMR and HRMS data of compounds 1–3, 15, and 16 and ECD spectra of compounds 1–3. [https://www.beilstein-journals.org/bjoc/content/supporting/1860-5397-17-151-S1.pdf]

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