Note

Inhibitory Effects of Polyacetylene Compounds from *Panax ginseng* on Neurotrophin Receptor-Mediated Hair Growth

Aoi Suzuki,[a] Daisuke Matsuura,[b] Hirotoshi Kanatani,[b] Shingo Yano,[b] Mitsuo Tsunakawa,[b] Shigeru Matsuyama,[c] and Hideyuki Shigemori*[,c]

[a] *Graduate School of Life and Environmental Sciences, University of Tsukuba; 1–1–1 Tennodai, Tsukuba, Ibaraki 305–8572, Japan:* [b] *Products Development Department, BATHCLIN CORPORATION; 29–9 Higashiarai, Tsukuba, Ibaraki 305–0033, Japan: and* [c] *Faculty of Life and Environmental Sciences, University of Tsukuba; 1–1–1 Tennodai, Tsukuba, Ibaraki 305–8572, Japan.*

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Neurotrophins play an important role in the control of the hair growth cycle. Therefore, neurotrophin receptor antagonists have therapeutic potential for the treatment of hair growth disorders. In this study, we investigated the inhibitory effect of *Panax ginseng*, a medicinal plant commonly used to treat alopecia, on the binding of neurotrophins to their receptors. In addition, we isolated and characterized the bioactive compounds of *P. ginseng* extracts. *P. ginseng* hexane extracts strongly inhibited brain-derived neurotrophic factor (BDNF)-TrkB and *β*-nerve growth factor (*β*-NGF)-p75 neurotrophin receptor (p75NTR) binding. Furthermore, we identified the following 6 polyacetylene compounds as the bioactive components in *P. ginseng* hexane extract: panaxynol (1), panaxydol (2), panaxydol chlorohydrin (3), 1,8-heptadecadiene-4,6-diyne-3,10-diol (4), panaxytriol (5), and dihydropanaxacol (6). In particular, compounds 4, 5, and 6 significantly inhibited BDNF-TrkB binding in a dose-dependent manner. To identify the structural component mediating the inhibitory effect, we investigated the effects of the hydroxyl moiety in these compounds. We found that the inhibitory effect of panaxytriol (5) was strong, whereas the inhibitory effect of Ac-panaxytriol (7) was relatively weak. Our findings suggest that *P. ginseng*-derived polyacetylenes with a hydroxyl moiety might provide therapeutic benefits to patients with hair growth disorders such as alopecia by inhibiting the binding of neurotrophins to their receptors. Although saponins have been proposed to be the primary mediators of the effects of *P. ginseng* on hair growth, this study revealed that polyacetylene compounds exert similar effects.

Key words *Panax ginseng*; polyacetylene; hair growth; brain-derived neurotrophic factor; nerve growth factor

The hair follicle exhibits a cyclical pattern of activity with alternating phases of rapid growth (anagen phase), apoptosis-driven regression (catagen phase), and relative quiescence (telogen phase).[1,2] In patients with androgenetic alopecia (AGA), the most common type of hair loss in men, the anagen phase is shortened and the telogen phase is extended.[3] 5*α*-Reductase plays a central role in the intrafollicular conversion of testosterone to dihydrotestosterone (DHT).[4] DHT binds to the androgen receptor with 5 times the avidity of testosterone and more potently activates downstream signaling events.[5] The binding of DHT to the androgen receptor induces the secretion of transforming growth factor-*β* (TGF-*β*), thereby suppressing the growth of hair follicle epithelial cells and disrupting the anagen phase.[6–8] A previous report demonstrated that brain-derived neurotrophic factor (BDNF) promotes the switch between the anagen and catagen phases by upregulating TGF-*β*.[9] Another report demonstrated that p75 neurotrophin receptor (p75NTR), a molecule that binds to *β*-nerve growth factor (*β*-NGF), plays a role in the regulation of apoptosis-driven hair follicle regression.[10] Neurotrophins exert their functional effects *via* interactions with high affinity receptors. Therefore, neurotrophin receptor antagonists have therapeutic potential for the treatment of hair growth disorders such as AGA.

Medicinal plants found in nature have long been used in conventional medicine for the treatment and prevention of diseases. In particular, Chinese medicinal plants are used to improve physical constitution. Extracts of Chinese medicinal plants contain an abundance of bioactive substances; however, the chemical structure and mechanisms mediating the bioactivity of some of these substances remains unknown.

Panax ginseng C. A. Meyer (*P. ginseng*) has long been used as a medicinal plant in East Asian countries and has been used to treat alopecia. Saponins are the primary bioactive substances of *P. ginseng*, and more than 20 types of ginsenosides and triterpene saponins have been isolated and structurally characterized.[11] Ginsenosides from *P. ginseng* have been reported to exert effects similar to those of minoxidil, the most commonly used topical drug for human hair growth.[12] Ginsenoside F2 induces hair growth by modulating the WNT signaling pathway.[13] Ginsenoside Rg3 also promotes hair growth.[14] Ginsenoside Ro has anti-androgen effects[15] and 5*α*-reductase has an inhibitory effect on hair growth.[16] However, the inhibitory effects of ginsenosides on the binding of neurotrophins to their receptors have yet to be reported.

In a preliminary experiment, we found that *P. ginseng* 70% EtOH extract (PG70-ext) and hexane extract (PGHE-ext) inhibit the binding of BDNF to TrkB. Although the synthetic small molecule ANA-12 has been shown to exert a similar effect,[17] there have been no previous reports describing the isolation and identification of natural inhibitors of BDNF-TrkB binding. The identification and structural characterization of such compounds is necessary for the development of a more effective treatment for AGA.

Therefore, we examined the inhibitory effect of compounds isolated from *P. ginseng* on BDNF-TrkB and *β*-NGF-p75NTR

* To whom correspondence should be addressed. e-mail: shigemori.hideyuk.fn@u.tsukuba.ac.jp

binding and evaluated their structure–activity relationships.

MATERIALS AND METHODS

Materials The root of *P. ginseng* with the rootlet was collected at Nagano Prefecture in 2007 and stored at 1°C prior to the experiments. The voucher specimen (BCPD-150401) is maintained at BATHCLIN CORPORATION.

Isolation and Identification of Polyacetylenes Dried and crushed root components (651 g) of *P. ginseng* were extracted by refluxing the sample with 1.3 L hexane 5 times. The solution was filtered, and the solvent was evaporated to yield 5.07 g of PGHE-ext. PGHE-ext (5.0 g) was purified using silica gel column chromatography (ϕ4.6×310 mm) (Nacalai Tesque Inc., Japan) and eluted using hexane–EtOAc (9 : 1→0 : 1) and MeOH to yield 12 fractions (PGHE1–12). PGHE2 (299 mg) was eluted with hexane–EtOAc (9 : 1) and purified using silica gel preparative thin layer chromatography (PLC) (1 mm, 20×20 mm) (Merck Millipore Inc., Japan) with hexane–acetone (9 : 1) to yield 9 subfractions (PGHE2-1–9). PGHE2-5 was identified as panaxynol[18] (**1**) (10.7 mg). PGHE4 (555 mg) was eluted with hexane–EtOAc (8 : 2) and purified using silica gel PLC with $CHCl_3$–MeOH (98 : 2) to yield 9 subfractions (PGHE4-1–9). PGHE4-5 was identified as panaxydol[19] (**2**) (13.7 mg). PGHE7 (82.8 mg) was eluted with hexane–EtOAc (7 : 3) and purified using silica gel PLC with $CHCl_3$–MeOH (99 : 1) to yield 11 subfractions (PGHE7-1–11). PGHE7-6 was identified as panaxydol chlorohydrin[19] (**3**) (16.6 mg). PGHE8 (114 mg) was eluted with hexane–EtOAc (7 : 3) and purified using silica gel PLC with $CHCl_3$–MeOH (96 : 4) to yield 11 subfractions (PGHE8-1–11). PGHE8-10 was identified as 1,8-heptadecadiene-4,6-diyne-3,10-diol[18] (**4**) (15.6 mg). PGHE10 (208 mg) was eluted with hexane–EtOAc (1 : 1) and separated into 4 subfractions (PGHE10-1–5) using silica gel PLC with $CHCl_3$–MeOH (96 : 4). PGHE10-5 was further purified using HPLC (TSKgel ODS-80Ts [ϕ4.6×250 mm], flow rate: 1.0 mL/min; MeCN–H_2O [1 : 9→7 : 3→1 : 0]; UV detection at 210, 254, and 280 nm) to yield 4 subfractions (PGHE10-5-1–4). PGHE10-5-2 and PGHE10-5-3 were identified as panaxytriol[19] (**5**) (3.70 mg, t_R 9.74 min) and dihydropanaxacol[20] (**6**) (1.30 mg, t_R 10.2 min), respectively. The structures of the compounds were confirmed using NMR (Avance 500, Bruker, Germany) spectral analyzes, and the findings were compared to previously published reports.[18–20]

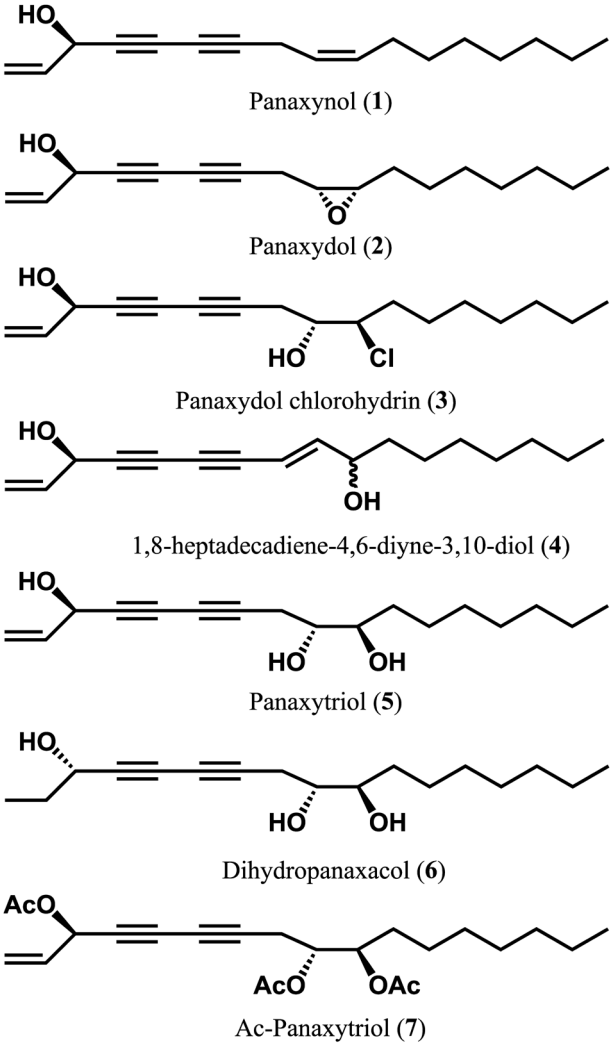

Fig. 1. Structures of the Isolated Compounds **1**–**6** from *P. ginseng* and Ac-Panaxytriol (**7**)

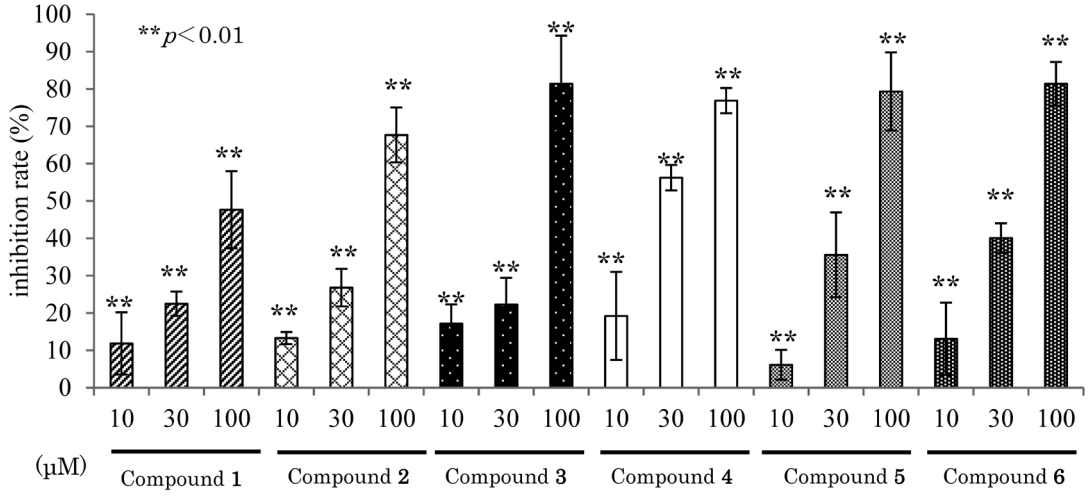

Fig. 2. Inhibitory Effects of Compounds **1**–**6** on the BDNF Binding to TrkB

These experiments were performed on the same plate. Each bar represents the mean±S.D. (n=3). **p<0.01.

Panaxytriol (5) Acetylation Panaxytriol (**5**) (0.5 mg: 1.8 nmol) was dissolved in 0.1 mL of dehydrated pyridine and 0.08 mL of acetic anhydride, and the mixture was incubated at room temperature for 5 h. The progression of the reaction was monitored using TLC. After 5 h, the mixture was evaporated using toluene to yield Ac-panaxytriol (**7**).

Ac-panaxytriol (**7**): GC-MS: *m/z* 404 (M^+), ^{1}H-NMR ($CDCl_3$) δ: 5.33 (1H, d, J=9.7 Hz, H-1a), 5.53 (1H, d J=17.5 Hz, H-1b), 5.86 (1H, ddd, J=17.5, 9.7, and 5.8 Hz, H-2), 5.90 (1H, d, J=5.8 Hz, H-3), 2.60 (1H, dd, J=17.5 and 6.2 Hz, H-8a), 2.58 (1H, dd, J=17.5 and 6.2 Hz, H-8b), 5.11 (1H td, J=6.2 and 4.2 Hz, H-9), 5.06 (1H, td, J=6.0 and 4.2 Hz, H-10), 2.12 (3H, s, OAc), 2.10 (3H, s, OAc), 2.09 (3H, s, OAc), 1.52 (2H, m, H-11), 1.55 (2H, m, H-12), 1.45–1.20 (8H, m, H-13, H-14, H-15, and H-16), and 0.88 (3H, t, J=7.0 Hz, H-17).

Neurotrophin Receptor Binding Inhibition Assay Binding inhibition assays to assess BDNF binding to TrkB and β-NGF binding to p75NTR were conducted at room temperature. Briefly, TrkB (R&D Systems, Minneapolis, MN, U.S.A.) or p75NTR (R&D Systems) was incubated in 96-well plates overnight at 4°C. Then, the wells were washed with phosphate buffered saline $(PBS)^-$ supplemented with 0.05% Tween 20, blocked with PBS^- supplemented with 5% Tween 20, 5% sucrose, and 0.05% NaN_3 for 1 h. The wells were washed again and subsequently incubated with sample solution (10, 30, and 100 μM) and 20 ng/mL recombinant human BDNF (Sigma, U.S.A.) or 10 ng/mL recombinant human β-NGF (Sigma) for 2 h. Then, the wells were washed and incubated with 100 ng/mL biotinylated anti-human BDNF (R&D Systems) or biotinylated anti-human β-NGF (R&D Systems) for 2 h. The wells were subsequently washed, incubated with HRP-conjugated streptavidin (R&D Systems) for 30 min, and washed again. The reactions were incubated with substrate solution for 15 min, and stop solution was subsequently added. The optical density at 405 nm in each well was measured.

Statistical Analysis Statistical differences between groups were determined using a Student's *t*-test, with $*p<0.05$ and $**p<0.01$ set as the level of significance.

RESULTS AND DISCUSSION

BDNF-TrkB binding inhibition was evaluated using 0.1 mg/mL of PG70-ext or PGHE-ext. PGHE-ext inhibited BDNF-TrkB binding by 89.2%, whereas PG70-ext inhibited BDNF-TrkB binding by 51.7%. As PGHE-ext does not contain saponins, we determined that saponins did not mediate the inhibitory effect.

The activity of PGHE-ext was determined using silica gel column chromatography, and PGHE-ext was further purified using silica gel PLC and reversed-phase HPLC. PGHE2-5, PGHE4-5, PGHE7-6, PGHE8-10, PGHE10-5-2, and PGHE10-5-3 were identified as panaxynol[18] (**1**), panaxydol[19] (**2**), panaxydol chlorohydrin[19] (**3**), 1,8-heptadecadiene-4,6-diyne-3,10-diol[18] (**4**), panaxytriol[19] (**5**), and dihydropanaxacol[20] (**6**), respectively. The structures of compounds **1**–**6** were confirmed using NMR spectral analyzes, and the results were compared to previously published reports[18–21] (Fig. 1).

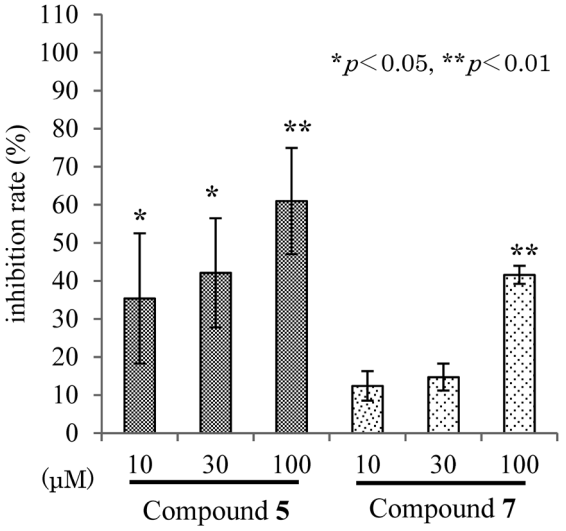

Fig. 3. Inhibitory Effects of Panaxytriol (**5**) and Ac-Panaxytriol (**7**) on the BDNF Binding to TrkB

These experiments were performed on the same plate. Each bar represents the mean±S.D. (n=3). $*p<0.05$, $**p<0.01$.

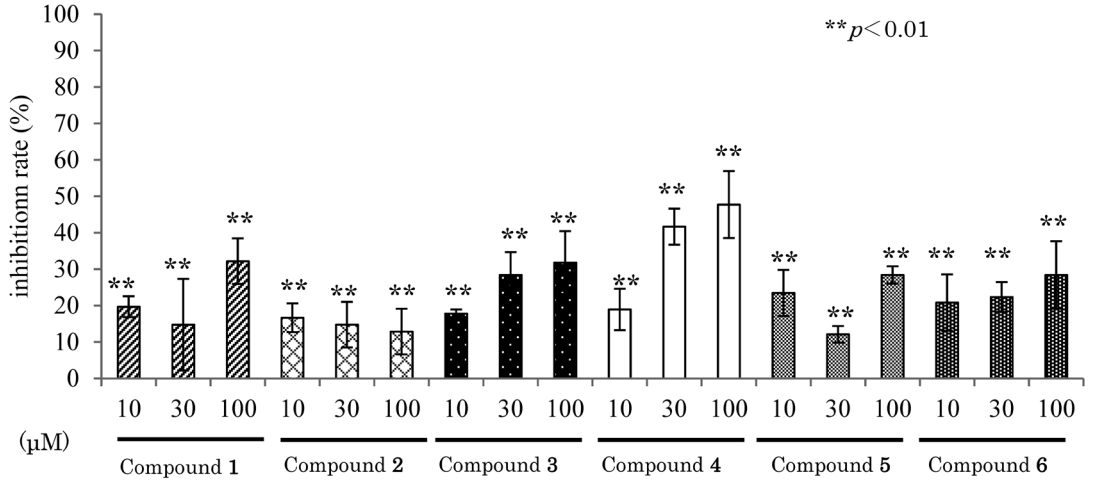

Fig. 4. Inhibitory Effects of Compounds **1**–**6** on the β-NGF Binding to p75NTR

These experiments were performed on the same plate. Each bar represents the mean±S.D. (n=3). $**p<0.01$.

The results of the inhibition assay demonstrated that compounds **1**–**6** inhibited BDNF-TrkB binding in a dose-dependent manner (Fig. 2). In particular, compounds 1,8-heptadecadiene-4,6-diyne-3,10-diol (**4**), panaxytriol (**5**), and dihydropanaxacol (**6**) exhibited stronger inhibitory activity compared with the other compounds, indicating that the number of oxygen function is a determinant of inhibition potency. To investigate this hypothesis, we evaluated and compared the inhibitory activity of panaxytriol (**5**) to the activity of its acetylated form (**7**) (Fig. 3). The inhibitory effect of Ac-panaxytriol (**7**) on BDNF-TrkB binding strongly decreased compared with panaxytriol (**5**), suggesting that the hydroxyl moiety of panaxytriol (**5**) is important for the inhibitory effect on BDNF-TrkB binding.

Compounds **1**–**6** slightly inhibited β-NGF-p75NTR binding (Fig. 4), while compounds **1**, **2**, and **5** did not show dose dependency. However, in contrast with the effects of compounds **1**–**6** on BDNF-TrkB binding, there was no clear structure–activity relationship associated with the inhibitory effect of the compounds on β-NGF-p75NTR binding.

A previous report indicated that BDNF promotes the switch between the anagen and catagen phases by upregulating TGF-β.[9] Furthermore, testosterone treatment has been reported to enhance BDNF production in mononuclear cells[22] and increase BDNF levels in male rats.[23] These findings indicate that neurotrophins such as BDNF play an important role in regulating the hair growth cycle. BDNF is also known to accelerate catagen progression by binding to TrkB in murine skin organ cultures.[24] p75NTR, a molecule that binds to β-NGF, has also been shown to play a role in hair growth by regulating apoptosis-driven hair follicle regression.[10] Neurotrophins exert their functional effects *via* interactions with high affinity receptors.[25] Therefore, neurotrophin receptor antagonists such as polyacetylene from *P. ginseng* have therapeutic potential for the treatment of hair growth disorders such as AGA.

In conclusion, this study demonstrated that polyacetylenes isolated from *P. ginseng* inhibit BDNF-TrkB binding. Furthermore, the structure–activity relationship of compounds **1**–**6** and **7** indicates that the number of hydroxyl moieties is important for their inhibitory effect on BDNF-TrkB binding. Saponins have been thought to be the primary mediators of the effects of *P. ginseng* on hair growth; however, this study demonstrated that polyacetylene compounds from *P. ginseng* exert similar effects.

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Conflict of Interest The authors declare no conflict of interest.

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