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Induction of Growth Hormone by the Roots of *Astragalus membranaceus* in Pituitary Cell Culture

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The traditional Asian medicinal herb, roots of *Astragalus* (*A.*) *membranaceus* (Leguminosae), is used for many purposes, some of which are purported to stimulate the release of growth hormone *in vivo*. Extracts of *A. membranaceus* were tested to determine whether they stimulate the release of growth hormone in rat pituitary cell culture. *A. membranaceus* was extracted sequentially with 80% ethanol (fraction A), *n*-hexane (fraction B); the test compound from the herbal extraction was isolated using silica gel column chromatography and was identified with spectral data. Test compound was also extracted by traditional boiling water methods. Induction of growth hormone in pituitary cell culture was conducted with isolated compounds and extracted fractions of *A.* Radix (dried roots of *A. membranaceus*). The fraction A was not active in the rat pituitary cell culture, but the fraction B derived from the ethanol fraction stimulated the release of growth hormone in culture. Six compounds from fraction B (**1-6**) were isolated and identified previously. The compounds 1,2-benzendicarboxylic acid diisononylester (**1**), β-sitosterol (**2**), and 3-*O*-β-D-galactopyranosyl-β-sitosterol (**5**) did not induce growth hormone release in the culture. Formononetin (**3**), 9*Z*,12*Z*-octadecadienoic acid (**4**), stigmast-4-en-6β-ol-3-one (**6**) and 98-E, a mixture of 1'-9,12-octadecadienoic acid (*Z,Z*)-2',3'-dihydroxy-propylester (**7**) and 1'-hexadecanoic acid-2',3'-dihydroxy-propylester (**8**) stimulated the release of growth hormone in the rat pituitary cell culture significantly compared to the control. In conclusions, four compounds isolated from extracts of *A.* Radix induced growth hormone release in the rat pituitary cell culture. The 98-E isolate was the most active inducer of growth hormone release.

Key words: *Astragalus membranaceus*, Leguminosae, Growth hormone, 1'-9,12-Octadecadienoic acid (*z,z*)-2',3'-dihydroxy-propylester, 1'-Hexadecanoic acid-2',3'-dihydroxy-propylester

INTRODUCTION

Astragalus (A.) membranaceus is one of the most important and popular herbs in traditional East-Asian medicine. It is grown in Korea, China (*A. chinensis* L.; *A. complanatus* R. Br.), Mongolia (*A. membramaceus var. mongholicus*), Japan (*Hedysarum iwawogi* Hara; *A. adsurgens* Pallas Subsp. fujisanensis Kita; *A. polybotrys* Hands-Mazz.), Egypt (*A. spinosus* Vahl; *A. trigonus*), and Bulgaria (*A. aitosensis*). It is used mainly as a tonic and diuretic (Li, 1982). It has been reported that the polysaccharide fraction of *A. membranaceus* stimulates depressed T-cell function in cancer patients (Geng, 1986). Triterpenglycosides and a flavonoide fraction of *A. membranaceus* have been reported to have antioxidant (Zhang *et al.*, 1992), antiinflammatory, and hypotensive activities (Harborne and Baxter, 1993; Tang, 1992).

Growth hormone (GH) is synthesized in somatotropes, pituitary acidophilic cells. GH release and production are controlled by the hypothalamic hormones, growth hormone releasing hormone (GRH) and somatostatin. Insulin-like growth factor-1 (IGF-1) mediates some GH regulation by peripheral feedback through GRH and somatostatin. GH is essential for postnatal growth as well as carbohydrate, lipid, nitrogen, and mineral metabolism (Finkenstedt *et al.*, 1997). GH promotes long bone growth at the epiphysial plates, increases formation of cartilage in children and stimulates appositional or acral growth in adults. Administration of GH prevents protein degradation in many diseases; it

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may delay physiological changes associated with aging (Horber and Haymond, 1990). Long-term treatment with GH increases lean body weight, muscle, and lipid weights (Kohrt *et al.*, 1992). Short-term treatment with GH increases plasma insulin and IGF-1 concentrations and decreases protein concentration in urine (Zachwieja *et al.*, 1994). GH stimulates liver activity and growth, and also increases protein synthesis in peripheral tissues, especially muscle, bone and connective tissues (Kaiser *et al.*, 1991; Marcus *et al.*, 1990). However, administration of human GH is limited by its poor absorption from the gastrointestinal tract and its high cost. Many studies are under way to overcome these inconveniences. Therapeutic options based on traditional Asian medicine may be an attractive alternative.

MATERIALS AND METHODS

Plant Materials

Roots of *A. membranaceus* were collected at Jungsun, Kangwon-do, Korea in 1995 and a voucher specimen (KIOM-95-3-0001) is stored at the herbarium of KIOM, Seoul, Korea.

Extraction and fractionation of n-hexane extract

Dried roots of *A. membranaceus* powder (2 kg) was extracted with 80% EtOH for 1 week at room temperature; this step was repeated four times (fraction A). Later, a *n*-hexane fractionation was conducted with fraction A to produce fraction B. The final water residue of fractionation was named fraction W. All reagents were of the highest grades available and were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) or from Merck Co. (Darmstadt, Germany). Concurrently, a traditional boiling water extraction was done at 90°C for 2.5 hrs (fraction T).

The active fraction B (*n*-hexane fraction) was applied to a silica gel 60 column (Merck Art. 9385, 7734) and eluted with $CHCl_3$: Me_2CO (= 9 : 1 - 1 : 1) and 100% EtOH, successively. TLC analysis was performed on precoated Kiesel gel 60 F_{254} (0.25 mm, Merck Art. 5715, 5729, 5789).

Isolation and identification of compounds

From fractionation of *n*-hexane extract, 1,2-benzendicarboxylic acid diisononylester (**1**), β-sitosterol (**2**), formononetin (**3**), 9z,12z-octadecadienoic acid (**4**), 3-*O*-β-D-galactopyranosyl-β-sitosterol (**5**), and stigmast-4-en-6β-ol-3-one (**6**) had been isolated from other plants and identified previously (Kim *et al.*, 1996; Kim and Kim, 1997). Expect for β-sitosterol (**2**) and formononetin (**3**), these compounds were isolated and identified from the *A. genus* for the first time (Fig. 1).

To obtain sufficient quantities, the *n*-hexane fraction (66 g) was fractionated in advance as reported in our previous studies (Kim *et al.*, 1996). The 19th sub-fraction (223 mg)

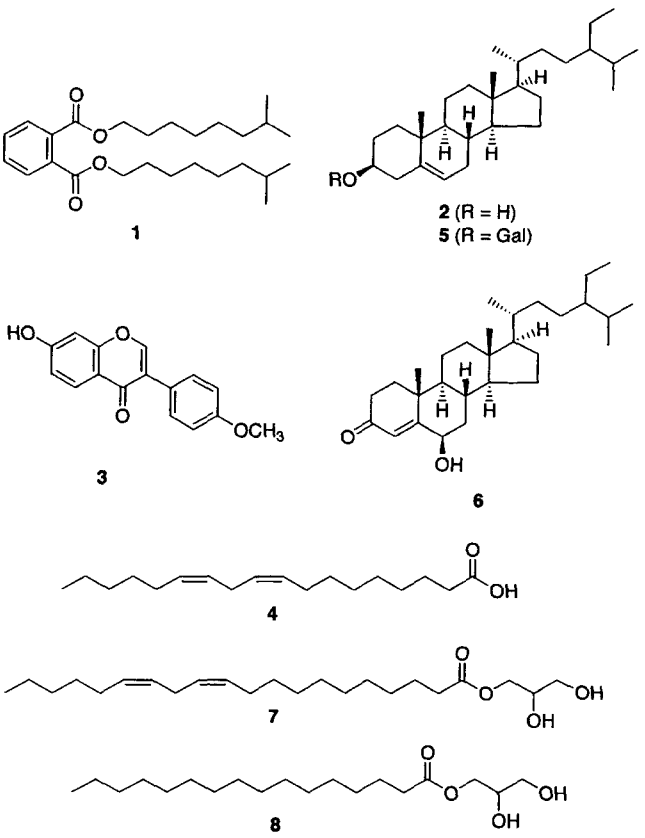

Fig. 1. Structures of isolated compounds **1**~**8**. **1**: 1,2-benzendicarboxylic acid diisononylester, **2**: β-sitosterol, **3**: formononetin, **4**: 9z,12z-octadecadienoic acid, **5**: 3-0-β-D-galactopyranosyl-β-sitosterol, **6**: stigmast-4-en-6β-ol-3-one, **7**: 1'-9,12-Octadecadienoic acid (z,z)-2',3'-dihydroxy-propylester, and **8**: 1'-Hexadecanoic acid-2',3'-dihydroxy-propylester

of the resultant 21 sub-fractions was again fractionated into 6 fractions (A1-6) using silica gel column chromatography (SGCC) (1.5 cm×56.5 cm, *n*-hexane : EtOH = 85 : 15, 0.2 ml/min). Then, SGCC (1.5 cm×17.5 cm, *n*-hexane : 2-PrOH = 9 : 1, 0.2 ml/min) (B1-5) was conducted again to further divide fraction A3 (106.5 mg) to isolate a colorless oily compound (98-E; 3.5 mg). In addition, fraction B3, which contained 98-E, was fragmented into four sub-fractions using SGCC (1.5 cm×16.0 cm, *n*-hexane : 2-PrOH = 9 : 1, 0.2 ml/min) (C1-4). The SGCC (1.5 cm×13.0 cm, *n*-hexane : 2-PrOH = 9 : 1, 0.1 ml/min) and preparative TLC were conducted to fractionate fraction C3 of the resultant four sub-fractions to isolate a compound 98-E (7.2 mg). Again further preparative TLC (n-hexane : 2-PrOH = 9 : 1) was conducted to C3 to isolate an additional 3.2 mg of 98-E, obtaining 10.7 mg of 98-E in total. Preparative TLC plates were cleaned twice with HPLC methanol and dried completely at 90°C before use in chromatography. 98-E is a colorless oily substance identified at UV_{254nm}; it turns violet when heat is applied after being sprayed with anisaldehyde-H_2SO_4.

Identification of compound, 98-E

***1'-9,12-Octadecadienoic acid (Z,Z)-2',3'-dihydroxy-propylester*: compound 7:** ^{1}H NMR, ^{1}H-^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.80-0.84 (3H, t, *J* = 7.0 Hz, H18), 1.18-1.60 (16H, s, H4-7, H14-17), 1.53-1.59 (2H, quintet, *J* = 7.0 Hz, H3), 1.94-2.01 (4H, q, *J* = 7.0 Hz, H8, 14), 2.03, 2.49 (each OH, br.s, disappear after D_2O addition), 2.26-2.29 (2H, t, *J* = 7.5 Hz, H2), 2.69-2.71 (2H, t, *J* = 6.5 Hz, H11), 3.51-3.55, 3.61-3.64 (each 1H, dd, *J* = 5, 10 Hz, H3'), 3.84-3.88 (1H, quintet, *J* = 5.0 Hz, H2'), 4.06-4.10, 4.12-4.15 (each 1H, dd, *J* = 5, 10 Hz), 5.25-5.33 (4H, m, H9, 10, 12, 13); ^{13}C NMR, DEPT, HMQC (125 MHz, $CDCl_3$) δ: 14.04 (C-18), 22.55, 29.08, 29.23, 29.43, 29.63, 31.52 (C4-7, C14-17), 24.89 (C-3), 25.63 (C-11), 27.17 (C-8, 14), 34.13 (C-2), 63.36 (C-3'), 65.18 (C-1'), 70.29 (C-2'), 127.90, 128.09, 130.00, 130.23 (C-9, 10, 12, 13), 174.32 (C-1); EI (+) MS (70 eV, *m/z*, relative intensity): 55, 98 (100), 134 (70), 239 (92), 257, 262, 299, 354 $[M]^+$.

***1'-Hexadecanoic acid-2',3'-dihydroxy-propylester*: compound 8:** ^{1}H NMR, ^{1}H-^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.80-0.84 (3H, t, *J* = 7.0 Hz, H16), 1.18-1.60 (24H, s, H4-15), 1.53-1.59 (2H, quintet, *J* = 7.0 Hz, H3), 2.03, 2.49 (each OH, br.s, disappear after D_2O addition), 2.26-2.29 (2H, t, *J* = 7.5 Hz, H2), 3.51-3.55, 3.61-3.64 (each 1H, dd, *J* = 5, 10 Hz, H3'), 3.84-3.88 (1H, quintet, *J* = 5.0 Hz, H2'), 4.06-4.10, 4.12-4.15 (each 1H, dd, *J* = 5, 10 Hz, H1'); ^{13}C NMR, DEPT, HMQC (125 MHz, $CDCl_3$): 14.08 (C16), 22.67, 29.12, 29.33, 29.57, 29.67 (C4-15), 24.91 (C3), 34.15 (C2), 65.18 (C1'), 70.29 (C2'), 63.36 (C3'), 174.33 (C1); EI (+) MS (70 eV, *m/z*, relative intensity): 98 (100), 134 (70), 238 [M-glyceryl group]$^+$ (25), 239 [M-glycerol]$^+$ (90), 330 $[M]^+$.

Preparation of pituitary cell culture and measurement of growth hormone release

Pituitary glands were aseptically removed from 30 young adult Sprague-Dawley male rats and rinsed in cold HBSS (Hanks Balanced Salt solution: Sigma Co., St. Louis, U.S.A.). The tissue was minced with a sterilized scalpel and was then suspended in three successive 10 ml aliquots of HBSS. The tissue suspension was then centrifuged at 1,200×g for 10 min and the pellet was resuspended in 30 ml of 0.2% collagenase (Life Technologies, Grand Island, NY, U.S.A.) and 0.2% hyaluronidase (Sigma Chem. Co., St. Louis, MO, U.S.A.) in HBSS at 37°C for 20 min. The enzyme digestion products were passed through a 70 μm nylon cell strainer (Becton Dickinson Labware, Franklin Lakes, NJ, U.S.A.) to remove undigested material. The pellet was resuspended in 15 ml culture medium. The culture medium consisted of containing 0.37% $NaHCO_3$, 10% horse serum, 2.5% fetal bovine serum, 1% nonessential amino acids, 1% glutamine, 1% nystatin, and 0.1% gentamycin (Life Technologies, Grand Island, NY, U.S.A.). One milliliter of the cell suspension (1.5×10^5 cells/ml) was placed in each well of a 24-well plate (Becton Dickinson Labware, Franklin Lakes, NJ, U.S.A.) and incubated (5% CO_2-95% air at 37 °C).

The cells were incubated for 4 days and then 100 μl of each samples or various concentrations of GRF were added. After 15 min incubation, the concentration of GH in the culture was quantified using the double antibody RIA method (Tanaka *et al.*, 1983, Wroblewski *et al.*, 1991). The GH standard curve was prepared from 4.55×10^{-9} M to 5.50×10^{-12} M. rGH assay kit (NIDDK, Harbor-UCLA medical center, CA, U.S.A.) containing ^{125}I-rat GH, rat GH antiserum (monkey), and rat GH were used. A second antibody (Goat IgG to monkey IgG: CAPPEL) and final 6% of PEG was added to precipitate the immuno-complex above and then the radioactivity of the precipitant (EG & G, Wallac, Finland) was counted; the content of GH in each sample was calculated from the regression of standard curve.

Induction of GH by all fractions (Table I) and isolated compounds including 1,2-benzendicarboxylic acid diisononylester (**1**), β-sitosterol (**2**), formononetin (**3**), 9Z,12Z-octadecadienoic acid (**4**), 3-O-β-D-galactopyranosyl-β-sitosterol (**5**), stigmast-4-en-6β-ol-3-one (**6**) and 98-E, a mixture of 1'-9,12-octadecadienoic acid (Z,Z)-2',3'-dihydroxy-propylester (**7**) and 1'-hexadecanoic acid-2',3'-dihydroxy-propylester (**8**) were measured by the same method.

Table I. GH assay results from Astragali Radix (Mean±SE: n=4)

Fraction	Concentration (mg/ml)	GH Concentration ($\times10^{-10}$ M)
Control	–	1.064 ± 0.038
A	2.7	1.386 ± 0.068
B	33.5	26.75 ± 1.886*
W	3.8	4.082 ± 0.796
T	–	1.014 ± 0.041

A: 80% ethanol extract.
B: n-Hexane extract.
W: final water residue of fractionation.
T: traditional boiling method extract..
*: Compared to control using ANOVA; P<0.01.

Statistics

All the results are expressed as the mean ± SE. The statistical significance between means was defined by ANOVA at P<0.05.

RESULTS

Fractionation of n-Hexane Extract of *A. membranaceus*

The yields of fraction A, B, and W from the extraction procedures were 316 g (15.8%), 29.4 g (1.47%), and 279 g

(14.0%), respectively. The bioactivities of these fractions are described as GH concentration ($\times10^{-10}$ M) released in the culture (Table I). Fraction B showed a greater than 20-fold increase in activity compared to the control.

The bioactivities of GH in each of the 21 fractions from n-hexane extract are reported as GH concentration ($\times10^{-10}$ M) released from rat pituitary cells grown in culture. Greater activity was observed with fractions 1, 11, 13, and 19 (Fig. 2).

Identification of Compound 98-E

98-E was isolated from fraction 19 in Fig. 2 and its structure was determined as following: ^{1}H NMR spectrum showed peaks of 1 terminal methyl group (δ 0.80-0.84, t, J = 7.0 Hz, H18), 4 olefin protons (δ 5.25-δ 5.33, m), 1 methylene group between olefin protons (δ 2.69-2.71, t, J = 6.5 Hz, H11), 2 methylene groups neighbor olefin protons (δ 1.94-2.01, quartet, J = 7.0 Hz, H8, 14), a methylene group neighbor a carboxyl group (δ 2.26-2.29, t, J = 7.5 Hz, H2), 8 methylene groups (δ 1.18-1.60, s), a methylene group at position 3 (δ 1.53-1.59, quintet, J = 7.0 Hz), and a carboxyl group (^{3}C: δ 174.32). From the finding that the peaks for the two methylene groups neighbor olefin protons were located between δ 25-28 of the ^{13}C NMR spectrum, it was determined that the olefin protons were *cis* double bonds. In addition, DEPT and HMQC analysis made it possible to estimate that the peaks at δ 63.36, 65.19 and 70.29 were those of glycerol. In other words, the δ 63.36 signal was linked to hydrogen shown at δ 3.51-3.55 (dd, J = 5, 10) and δ 3.61-3.64 (dd, J = 5, 10); protons as indicated by the δ 65.19 signal, to hydrogen shown at 4.06-4.10 (dd, J = 5, 10); and the δ 70.29 signal, to hydrogen shown at 3.84-3.88 (quintet, J = 5 Hz). It was also determined through homo-COSY that methine protons were coupled with methylene protons on both sides to express themselves in the form of a quintet. The finding that the methylene group at position 1 of glycerol was closer to the lower magnetic field than the methine group shows that an unsaturated fatty acid was bound to position 1.

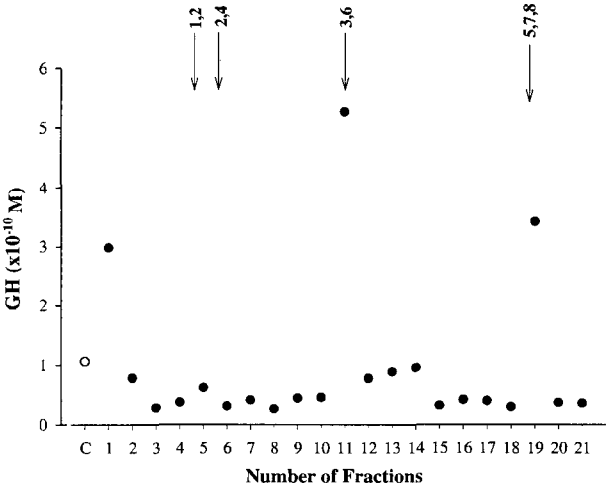

Fig. 2. Growth hormone activity as measured by chromatography. X-axis is number of fraction after the silica gel column chromatography and Y-axis is GH activity ($\times10^{-10}$ M) by RIA. Each point indicates mean of triplicate assay. The arrows indicate the fraction of each compounds in Fig. 1 isolated.

In EI (+) MS spectrum, a molecular ion peak appeared at *m/z* 354, and a fragment ion peak was also identified at *m/z* 134 (70%), which was a result of Mclafferty reaction, characteristic of ester compounds (Hess *et al.*, 1991). The spectrum also showed peaks at *m/z* 116 (15%) and 98 (100%) for molecular ions formed with one H_2O molecule and two H_2O molecules removed. These peaks of OH groups disappeared through D_2O exchange. In addition, peaks at *m/z* 262 (65%) and 265 (28%), which were molecular ions formed after the glyceryl group and gycerol group were removed, confirmed the existence of gycerol. These data on fatty acids matched the NMR library spectrum data except for those for linoleic acid (9Z,12Z-octadecadienoic acid) and carboxylic acid (Pouchert and Behnke, 1993a). Analysis of all these data confirmed that the substance was 1'-9,12-octadecadienoic acid (Z,Z)-2',3'-dihydroxy-propylester (1-monolinolein).

In NMR spectrum, peaks except for those of olefin protons and hydrogens at H8, 11 and 14 showed integrals twice as larger, indicating that compounds binding saturated fatty acids existed mixed with glycerol. And the data on fatty acids matched NMR library spectrum data except for those on palmitic acid (hexadecanoic acid) and carboxylic acid (Pouchert and Behnke, 1993b). EI (+) MS spectrum also showed $[M]^+$ 330 representing $C_{19}H_{38}O_4$; peaks at *m/z* 238 (25%) and 239 (90%) for ions formed with the glyceryl and gycerol groups removed; and a peak at *m/z* 134 (70%) for a fragment ion indicating that the substance is a ester compound. Analysis of all these data confirmed that the substance was 1'-hexadecanoic acid-2',3'-dihydroxy-propylester.

Measurement of GH activity in rat pituitary cell culture

The concentrations of GH released in the rat pituitary cell culture were correlated to those of GRF added in Fig. 3. 1,2-benzendicarboxylic acid diisononylester (**1**) ((1.25 ± 0.20)$\times10^{-10}$ M, n = 3), β-sitosterol (**2**) ((0.93 ± 0.22)$\times10^{-10}$ M, n = 8), and 3-*O*-β-D-galactopyranosyl-β-sitosterol (**5**) (0.63 ± 0.00)$\times10^{-10}$ M, n = 2) did not induce GH in the culture compared to control ((1.06 ± 0.04)$\times10^{-10}$ M, n = 12). Formononetin (**3**) ((2.08 ± 0.42)$\times10^{-10}$ M, n = 6), 9Z,12Z-octadecadienoic acid (**4**) ((2.49 ± 0.52)$\times10^{-10}$ M, n = 6) and stigmast-4-en-6β-ol-3-one (**6**) ((2.45 ± 0.43)$\times10^{-10}$ M, n = 2) stimulated the release of GH in rat pituitary cell culture (Fig. 4). 9Z,12Z-octadecadienoic acid (**4**) and stigmast-4-en-6β-ol-

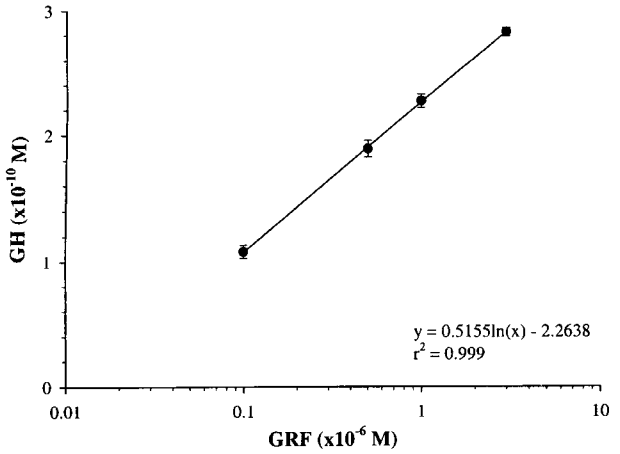

Fig. 3. A representative standard curve of GH released by GRF in the rat pituitary cell culture. X-axis is various concentrations of GRF and Y-axis is GH released. The fitting equation is Y = 0.5156 ln(X) – 2.2638 (R^2 =0.999).

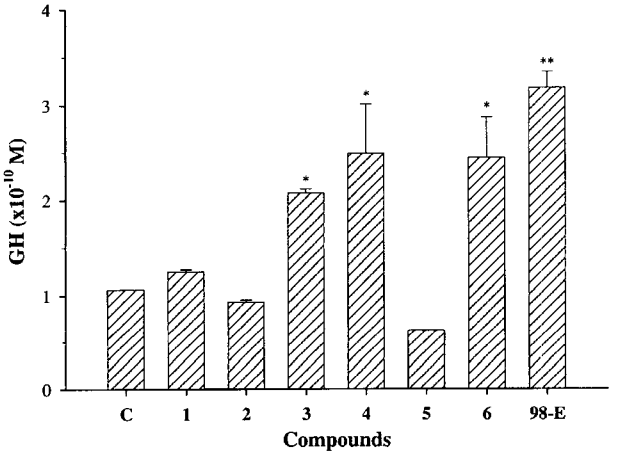

Fig. 4. Induction of growth hormone activity by isolated compounds from Astragali Radix. X-axis is name of compound isolated and Y-axis is GH activity ($\times 10^{-10}$ M) by RIA. 10 μg/ml of sample was used for the assay. Each compound indicates a bar as a mean of 3-12 samples and error bar means standard error of mean. Compounds are **C**: control, **1**: 1,2-benzendicarboxylic acid diisononylester, **2**: β-sitosterol, **3**: formononetin, **4**: 9z,12z-octadecadienoic acid, **5**: 3-0-β-D-galactopyranosyl-β-sitosterol, **6**: stigmast-4-en-6β-ol-3-one and **98-E**: a mixture of 1'-9,12-Octadecadienoic acid (z,z)-2',3'-dihydroxy-propylester and 1'-Hexadecanoic acid-2',3'-dihydroxy-propylester. *P<0.05, **P<0.01; both compared to control by ANOVA.

3-one (**6**) were active inducers of GH released in the culture. 98-E was the most active inducer of GH release ($(3.18 \pm 0.16)\times 10^{-10}$ M, n = 4) (Fig. 4).

Abbreviations GH: Growth Hormone, rGH: rat Growth Hormone, GRF: Growth Hormone Releasing Factor, GRH: Growth Hormone Releasing Hormone, IGF-1: Insulin-like Growth Factor-1, IgG: Immunoglobulin G, SCC: Silica Gel Column Chromatography, homo-COSY: homo Correlation Spectroscopy, DEPT: Distortionless Enhancement by Polarization Transfer, HMQC: Heteronuclear Multiple Quantum Coherence, EI (+) MS: Electron Impact (+) Mass Spectrum, *m/z*: mass fragmentation, br.s: broad singlet, HBSS: Hanks Balanced Salt solution, DMEM: Dulbecco's Modified Eagle Medium, 98-E: mixture of 1'-9,12-Octadecadienoic acid (*z,z*)-2',3'-dihydroxy-propylester and 1'-Hexadecanoic acid-2',3'-dihy- droxy-propylester, SE: Standard Error of mean, ANOVA: Analysis of Variance.

DISCUSSION

The roots of *A. membranaceus* (Leguminosae) comprise nearly 10% of total medicinal herbs in East-Asian market and have been used widely as tonics. Some of the traditional uses of the roots of *A. membranaceus* are similar to therapeutic uses of GH as increases in wound healing and strength of muscles and bones. For the first time, the induction of growth hormone release from a rat pituitary cell culture stimulated by preparations from *A. membranaceus* are reported.

Following sequential extraction and fractionation, the test compounds were incubated in a rat pituitary cell culture and the bioactivities of the extracts were measured by an assay developed earlier in our laboratory (Kim *et al.*, 2000). Bioactivity of each fraction were normalized to the amount used for the assay; the normalized results showed that n-hexane fraction was very active and had a high yield. Formononetin (**3**) and 9Z,12Z-octadecadienoic acid (**4**) were very active inducers of GH release in pituitary cell culture. Formononetin (**3**), a well-known phytoestrogen, has hypolipidemic properties (Siddiqui and Siddiqui, 1976), acts like estrogen (Ruh *et al.*, 1997), and is a carcinogen in mammary gland (Wang *et al.*, 1995). 9z,12z-octa decadienoic acid (**4**) is an essential fatty acid that synthesizes eicosanoic fatty acid, which is important physiologically and pharmacologically as it regulates the formation of prostaglandins, thrombaxanes, leukotrienes, and lipoxins. Stigmast-4-en-6β-ol-3-one (**6**) has been found in *Typha latifolia* (Greca *et al.*, 1990) and *Calotropis procera* (Khan and Malik, 1989), but its biological activity has not been reported.

Analysis of all the ^{1}H NMR, ^{13}C NMR, homo-COZY, DEPT, HMQC, EI (+) MS data confirmed that the compound 98-E was a mixture of 9,12-octadecadienoic acid (*z,z*)-2',3'-dihydroxy-propylester (1-monolinolein) and 1'-hexadecanoic acid-2',3'-dihydroxy-propylester. These compounds are inseparable fatty acids ethylesters. They were isolated in Astragalus genus for the first time here; beta-monolinolein was isolated from *Bulperum sibirium* previously (Song *et al.*, 1993). One report found them to be effective to reduce cholesterol levels in blood (Kobayashi *et al.*, 1984).

None of these compounds have been reported previously to induce release of GH in cell culture and the mecha-

nisms mediation this release cannot be explained from this research. Further systemic fractionations and isolations of other active compounds derived from *A. membranaceus* and their effects on GH release are in progress.

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REFERENCES

Finkenstedt, G., Gasser, R. W., Hofle, G., Watfah, C. and Fridrich, L., Effects of growth hormone (GH) replacement on bone metabolism and mineral density in adult onset of GH deficiency: results of double-blind placebo-controlled study with open follow-up. *Eur. J. Endocrinol.*, 136, 282-289 (1997).

Geng, C. S., Advances in immuno-pharmacological studies on Astragalus membranaceus. *Chung His I Chieh Ho Tsa Chih Chin. J. Mod. Develop. Trad. Med.*, 6(1), 62-4 (1986).

Greca, M. D., Monaco, P. and Previteva, L., Stigmasterols from *Typha latifolia*. *J. Nat. Prod.*, 53, 1430-1435 (1990).

Harborne, J. B. and Baxter, H., Phytochemistry Dictionary. Taylor & Francis, London, pp. 673, (1993).

Hess M, Meier, H. and Zeeh, B., Spektroskopische Methoden un der organischen Chemie. Georg Thieme Verlag, Stuttgart, pp. 240-241, (1991).

Horber, F. F. and Haymond, M. W., Human growth hormone prevents the protein catabolic side effects of prednisone in humans. *J. Clin. Invest.*, 86, 265-272 (1990).

Kaiser, F. E., Silver, A. J. and Morley, J. E., The effect of recombinant human growth hormone on malnourished older individuals. *J. Am. Geriat. Soc.*, 39, 235-240 (1991).

Khan, A. Q. and Malik, A., A Steroid from Calotropis procera. *Phytochemistry*, 28, 2859-2861 (1989).

Kim, C., Kim, J. S. and Park, S. J., Use of 7-Hydroxy-4'-methoxy isoflavone and its derivatives for releasing growth hormone. *Korean Patent No.* 0251519, (2000).

Kim, J. S. and Kim, C., A Study on the Constituents from the Roots of *Astragalus membranaceus* (II). *Kor. J. Pharmacognosy*, 28(2), 75-79 (1997).

Kim, J. S., Kim, Y. T. and Kim, C., A Study on the Constituents from the Roots of Astragalus membranaceus (I). *Kor. J. Pharmacognosy*, 27(4), 336-341 (1996).

Kobayashi, T., Sekine, K., Mackawa, Y., Suzuki, H., Shimoda, Z. and Ono, I., Additives for foods and pharmaceuticals. *European Patent Apply No.* EP 104043 A2, (1984).

Kohrt, W. M., Maily, M. T., Dalsky, G. P. and Holloszy, J. O., Body composition of healthy sedentary and trained, young and older men and women. *Med. Sci. Sports Exercise*, 24, 832-837 (1992).

Li, S. Z., Ben Cao Gang Mu (Compendium of Materia Medica). Ren Min Wei Sheng Publishing Co., Beijing, China, pp. 696, (1982).

Marcus, R., Butterfield, G., Holloway, L., Gilliland, L., Baylink, D. J., Hintz, R. L. and Sherman, B. W., Effects of short term administration of recombinant human growth hormone to elderly people. *J. Clin. Endocrinol. Metab.*, 70, 519-527 (1990).

Pouchert, J. B. and Behnke, J., The Aldrich Library of 13C and 1H FTNMR spectra. 1, pp. 756B, (1993).

Pouchert, J. B. and Behnke, J., The Aldrich Library of 13C and 1H FTNMR spectra. 2, pp. 785B, (1993).

Ruh, M. F., Taylor, J. A., Howlett, A. C. and Welshons, W. V., Failure of cannabinoid compounds to stimulate estrogen receptors. *Biochem. Pharmacol.*, 53(1), 35-41 (1997).

Siddiqui, M. T. and Siddiqui, M., Hydrolipidemic principles of Cicer arietinum: biochanin A and formononetin. *Lipids.*, 11(3), 243-246 (1976).

Song, Z., Jia, Z. and Zhu, Q., Study on the components of Bupleurum sibiricum. Lanzhou Daxue Xuebao. *Ziran Kexueban*, 29(2), 85-89 (1993).

Tanaka, T., Shishiba, Y., Gout, D. W., Beer, C. T., Noble, R. L. and Friesen, H. G., Radioimmunoassay and bioassay of human growth hormone and human prolactin. *J. Clin. Endocrinol.*, 56(1), 18-20 (1983).

Tang, W. and Eisenbrand, G., Chinese Drugs of Plant Origin. Springer-Verlag, Berlin, pp. 191, (1992).

Wang, W., Tanaka, Y., Han, Z. and Higuchi, C. M., Proliferative response of mammary glandular tissue to formononetin. *Nutrit. Cancer*, 23(2), 131-140 (1995).

Wroblewski, V. J., Masnyk, M. and Becker, G. W., Proteolytic cleavage of human growth hormone (hGH) by rat tissues in vitro: influence on the kinetics of exogenously administered hGH. *Endocrinology*, 129(1), 465-474 (1991).

Zachwieja, J. J., Bier, D. M. and Yarasheski, K. E., Growth hormone administration in older adults: effects on albumin synthesis. *Am. J. Physiol.*, 266: (*Endocrinol. Metab.* 29), E840-844 (1994).

Zhang, Y. D., Shen, J. P., Zhu, S. H., Huang, D. K., Ding, Y. and Zhang, X. L., Effects of Astragalus on experimental liver injury. *Acta. Pharmacologica. Sinica.*, 27(6), 401-406 (1992).