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여러가지 조건하에서 재배한 바위솔에서 스테로이드와 테트라싸이클릭 트리테르펜의 가스 크로마토 그래피를 이용한 분석

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Determination of Steroids and Tetracyclic Triterpenes in *Orostachys japonicus* A. Beger Grown under Various Cultivation Conditions Using Gas Chromatography

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ABSTRACT : The content of two steroids-campesterol (1) and β-sitosterol (2), and four triterpenes-taraxetrone (5), β-amyrin (6), (–)-friedelin (7), glutinol (8) in the *Orostachys japonicus* A. Berger cultivated under various conditions was estimated and compared with those in wild one. The present investigation disclosed that there are no significant difference in their contents between cultivated *Orostachys japonicus* A. Berger and wild one. From viewpoint of the content of the steroids 1 and 2, and the triterpenes 5-8, the quality of cultivated *Orostachys japonicus* A. Berger is not inferior to the wild one.

Key Words: : *Orostachys japonicus* A. Beger, Steroids, Triterpenes, GC

INTRODUCTION

Orostachys japonicus A. Berger is a plant native in eastern Asia and distributed widely in Korea and China. The extract of *Orostachys japonicus* A. Berger is frequently used in folk cancer remedy and health food in Korea, where patients and peoples drink hot water extract of the plant (Kang *et al.*, 2005a; Kang *et al.*, 2005b). Recently, the consumption of the *Orostachys japonicus* A. Berger had increased greatly due to the increase in the number of cancer patients in Korea. Therefore, cultivating the *Orostachys japonicus* A. Berger is attractive to farmers as an important income producing crop. However, since both the cultured *Orostachys japonicus* A. Berger and wild one are released into the market at the same time (i.e. early October), the market price plummet. In order to prevent a sharp drop in the price, the time for releasing the plant to market needs to be controlled (Kang *et al.*, 1995; Kang *et al.*, 1996; Kang *et al.*, 1997).

Recently, Kang *et al.* (1997) examined the retardation of *Orostachys japonicus* A. Berger growth under the various conditions of cultivation such as a night-break treatment, a day-length adjustment and a temperature control during the winter season. They showed that regulating such cultivation conditions retards the growth of the *Orostachys japonicus* (Kang *et al.*, 1995; Kang *et al.*, 1996; Kang *et al.*, 1997). It means that the cultured *Orostachys japonicus* A. Berger can be released into market at the farmer's discretion. However, it is important to retain quality of the cultured *Orostachys japonicus* A. Berger. Thus, the content of the health promoting components should not be lower than in the wild one. In order to disclose the quality of the

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cultured *Orostachys japonicus* A. Berger, we investigated the content of phenolic compounds and compared it with the wild type plant. The phenolic compounds are occurring in food plants and exhibit a wide range of biological effects, including antibacterial, anti-inflammatory, antiallergic, anticalcinogenic action and reduction of coronary heart disease (Merken *et al.*, 2000; Harborne *et al.*, 2000).

Very recently, the content of some steroids and tetracyclic triterpenes in *Orostachys japonicus* A. Berger samples collected from day-length control, night-break and fertilization experiments was analyzed by gas chromatography and mass spectroscopy. Steroids and tetracyclic triterpenes are classes of naturally occurring substances widely distributed in the plant kingdom and in some marine organisms. Steroids and tetracyclic triterpenes play an important role as constituents of cell membranes, insect deterrents and growth hormone and used in traditional medicine and some of these, like cardiac glycosides such as digoxine and digitoxin originated from Digitalis, are important and valuable drugs (Zeelen *et al.*, 1995; Shimada *et al.*, 2001).

MATERIALS AND METHODS

1. Cultivation

Seedlings of the *Orostachys japonicus* A. Berger were transplanted into a greenhouse in the experimental farm at Gyeongsang National University on May 27, 2005. Each seedling was assigned to a different plastic pot of 18 cm diameter, which was filled with a soil and fertilizer blend (2 : 1, v/v). Sufficient water was supplied to the *Orostachys japonicus* A. Berger every two or three days, and the ambient temperature was retained throughout the experiments. Any agricultural chemicals were not scattered.

2. Light conditions

The *Orostachys japonicus* A. Berger was divided to two sample groups, and the night-break experiment was carried out on each sample group from on July 2 and August 25, 2005. The sample was irradiated with 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of light for two hours from 11 p.m. to 1 a.m. using an incandescent electric lamp. The *Orostachys japonicus* A. Berger samples were gathered before bursting into bloom. Thus, the sample planted at July 2 was collected on September 4, and one planted at August 25 were collected on October 6.

3. Day-length control

The *Orostachys japonicus* A. Berger was divided to two sample groups, and the day-length control experiment was carried out on each sample group from on June 20, 2005, according to the following day-length: 13/7 and 16/8 h (day/night) employing a light-curtain. An incandescent electric lamp with a luminosity 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was used as the light source. The day-lengths 13 and 16 h, were from 8 a.m. to 9 p.m. and from 5 a.m. to 9 p.m., respectively. The *Orostachys japonicus* A. Berger samples were gathered before bursting into bloom. Thus, samples of the 13/7 and 16/8 h plants were collected on October 25 and on November 4, respectively.

4. Brightness control

The *Orostachys japonicus* A. Berger was divided to three sample groups, and the brightness control experiment was carried out on each sample group from on June 20 to November 12, 2005. The sun light was shielded on each sample to 0, 35 and 55% by light-curtain. The *Orostachys japonicus* A. Berger samples were gathered on November 12.

5. Fertilization

The *Orostachys japonicus* A. Berger was divided to three sample groups, and the nitrogen, phosphorous (P_2O_5), and potassium (K_2O) fertilizer were scattered on each sample in amount of 5, 10 and 20 kg/ha on May 27, 2005. The samples were gathered on November 12.

6. Preparations of chemicals

The wild *Orostachys japonicus* A. Berger was purchased at the Chinju Central Market on October 1, 2005. Organic solvents with analytical reagent grade and silica gel plates for prep-thin layer chromatography were all purchased from Merk Co. (Darmstadt, Germany). Two steroids-campesterol (**1**), β-sitosterol (**2**), and five triterpenes-taraxetrone A (**5a**), taraxetrone B (**5b**), β-amyrin (**6**), (–)-friedelin (**7**), glutinol (**8**) as standard materials were isolated from wild *Orostachys japonicus* A. Berger in our laboratories by literature methods (Park *et al.*, 1994; Park *et al.*, 1991a; Park *et al.*, 1991b). The purity of the isolated reference materials were shown to be higher than 99% analyzed by GC, and their identities were confirmed by IR, ^1H- and ^{13}C-NMR, and mass spectroscopy.

7. Analytical methods

GC analysis was performed using 6890N network GC system (Agilent Technologies) equipped with a flame ionization detection (FID) system. Data were recorded and analyzed by HP3396 series 2 integrator. A Supelco SAC-5 capillary column (30 m × 0.25 ㎛) was employed. Nitrogen was used as a carrier gas at a flow-rate 20 ㎝ · sec^{-1}. Oven temperature was set at 265℃, and both injector and detector was operated at 300℃. An aliquot (1.0 μL) of sample was injected with a split with a split injection of 25 : 1.

Chloroform stock solutions containing campesterol (**1**), β-sitosterol (**2**), and five triterpenes-taraxetrone A (**5a**), taraxetrone B (**5b**), β-amyrin (**6**), (−)-friedelin (**8**), glutinol (**6**) were prepared, respectively and then diluted to appropriate concentration range for the construction of calibration curves. Aliquots (1.0 ㎕) of the sample were then injected into GC system. Each calibration curve was performed in triplicate with five different concentrations. The concentration of the internal standard, octacosane was 60 ㎍ · mL^{-1} for all analyses. Calibration curves were constructed by plotting peak area ratio (analyte/internal standard) versus.

8. Analysis of seven components in *Orostachys japonicus* A. Bergers

Freeze-dried and grounded *Orostachys japonicus* A. Berger samples (1.0 g) were added into the internal standard solution (10.0 mL). The mixtures were extracted by homogenizer (Nissei AM-7, Nihonseiki kaisha LTD.) for 5 min at 10,000 rpm. The extracts were filtered using filter paper (Whatman No1). Aliquots (1.0 μL) of the solutions were directly subjected to GC analysis. The contents of the analysys determined from the corresponding calibration curves.

RESULTS AND DISCUSSION

By now, four steroids **1-4** and five triterpenes **5-9** in the *Orostachys japonicus* A. Berger have been known (**Fig. 1**) (Park *et al.*, 1994; Park *et al.*, 1991a; Park *et al.*, 1991b). In this study, two steroids-campesterol (**1**) and β-sitosterol (**2**), and four triterpenes-taraxetrone (**5**), β-amyrin (**6**), (−)-friedelin (**7**), glutinol (**8**) were isolated from the wild *Orostachys japonicus* A. Berger according to the literatural method (Park *et al.*, 1994; Park *et al.*, 1991a; Park *et al.*, 1991b) and used as standard materials to evaluate the quality of the *Orostachys japonicus* A. Berger cultivated under various conditions.

Since steroids and triterpenes containing at least one polar hydroxyl function group cannot be eluted from conventional GC Column a GC analytical method with pre-column derivatization has been widely utilized (Shimada *et al.*, 2001). It is time consuming and requires completion of the derivatization in order to achieve accuracy of quantification. Supelco SAC-5 (30 ㎝ × 0.25 ㎜, 0.25 ㎛) (Sigma-Aldrich Korea) which has been specifically designed for the analysis

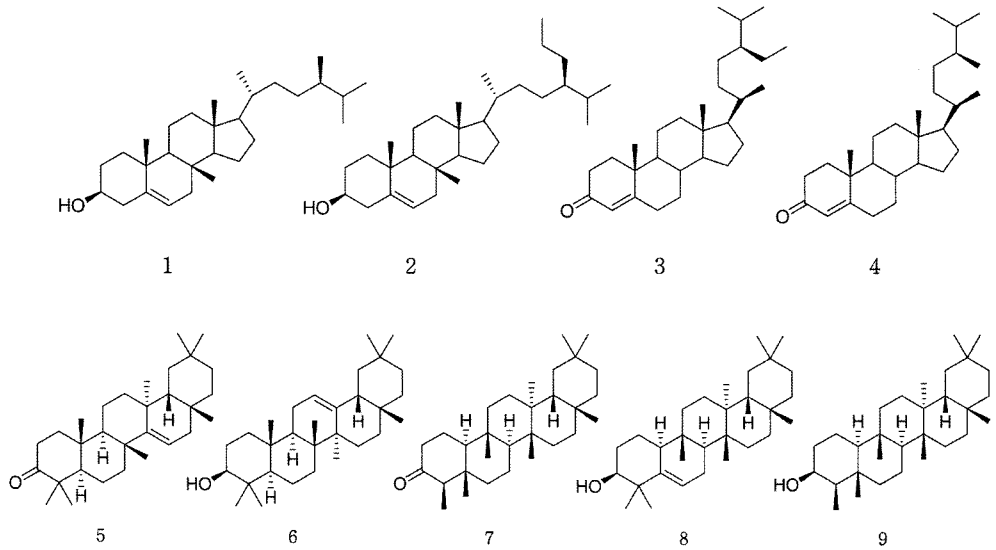

Fig. 1. Steroids-campesterol (**1**) and β-sitosterol (**2**), stigmast-4-en-3-one (**3**), ergost-4-en-3-on (**4**) and triterpenes-taraxetrone (**5**), β-amyrin (**6**), (−)-friedelin (**7**), glutinol (**8**), epifriedelanol (**9**) were isolated from the wild *Orostachys japonicus* A. Berger.

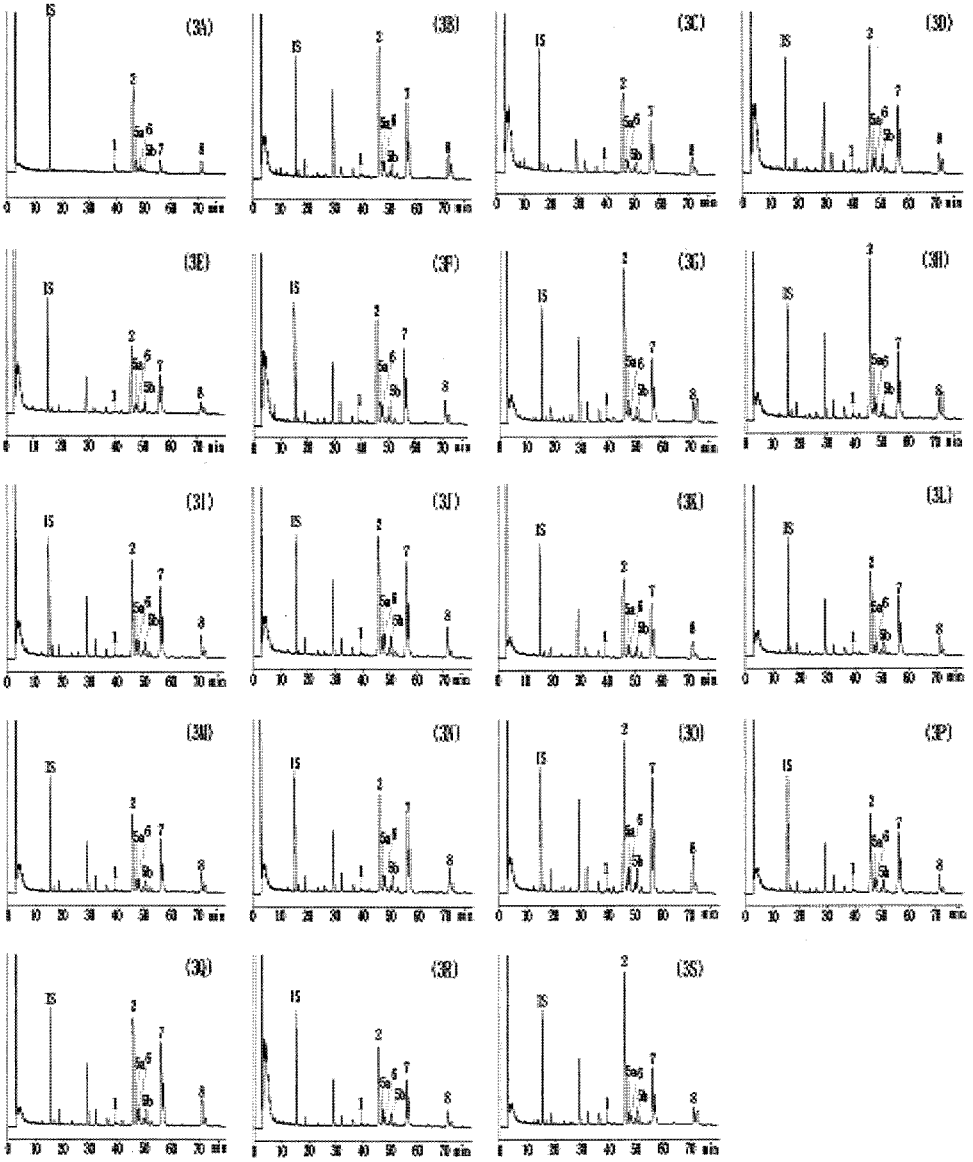

Fig. 2. Gas chromatogram of the extracts of the *Orostachys japonicus* A. Berger cultivated under various conditions. (3A): Standard Material, (3B): Night-break 7/2, (3C): Night-break 8/25, (3D): Day-length 13:11, (3E): Day-length 16:08, (3F): Brightness 0%, (3G): Brightness 35%, (3H): Brightness 55%, (3I): Nitrogen Fertilizer N5, (3J): Nitrogen Fertilizer N10, (3K): Nitrogen Fertilizer N20, (3L): Potassium Fertilize K5, (3M): Potassium Fertilize K10, (3N): Potassium Fertilize K20, (3O): Phosphorus Fertilizer P5, (3P): Phosphorus Fertilizer P10, (3Q): Phosphorus Fertilizer P20, (3R): Before Blooming of wild, (3R): After Blooming of wild.

of steroids was used for the study. It allowed to resolve steroids **1** and **2**, and triterpenes **5-8** well with base-separations without derivatization (**Fig. 2**).

Calibration curves were constructed by plotting peak area ratio (analyte/internal standard) versus concentration of the analyte. The six calibration curves all showed good linear regression as summarized in **Table 1**.

Gas chromatograms of the extracts of the *Orostachys japonicus* A. Berger cultivated under various conditions are shown in **Fig. 2**, and the quantity of each steroid and triterpene identified are summarized in **Table 2**.

As shown in **Table 2**, in all of the investigated *Orostachys japonicus* A. Berger the steroid **2** was contained much more than **1**. The content of **1** in the wild *Orostachys japonicus* A. Berger before blooming was compared with that after blooming. The content of **2** before blooming was higher than that after blooming. In the luminescence variation experiments the brightness control

(55%) showed the highest contents of **1** and **2**. In contrast, the lowest content of **1** and **2** were observed at day-length control (16:08). In the fertilization experiments, K5 and K20 showed the highest content of **1** and **2**. These results demonstrated that there is an clear linear relationship between production of the steroids **1** and **2** and amount of added light. Light would be somewhat unfavorable or in effective to the biosynthesis of the steroids **1** and **2**. A variety and amount of fertilization are also likely not to be concerned to their biosyntheses.

As shown in **Table 2**, of all four triterpenes **5-8**, the content of **7** was highest in the *Orostachys japonicus* A. Berger investigated and it of **6** was lowest. The content of **5** in the wild *Orostachys japonicus* A. Berger before blooming was higher than that after blooming. The contents of **6** and **8** before blooming were comparable to those after blooming. In the luminescence variation experiments It was found that the content of **5** was highest at brightness control (36%) and lowest at day-length control (16 : 8). The contents of **6-8** were highest at night-break 7/2. While the

Table 1. Calibration curves and r^2 values of the standard materials **1**, **2** and **5-8**

Standard material	Retention time (RT, min)	Calibration curve[a]	r^2
1	39.33	y = 0.3029x + 0.6175	0.995
2	46.36	y = 0.6876x + 13.3784	0.995
5	47.71	y = 0.3216x – 0.8612	0.999
6	48.38	y = 0.3478x + 1.0801	0.997
7	56.51	y = 0.1731x + 0.4361	0.999
8	71.75	y = 0.6340x + 0.8968	0.999

[a] y: peak area ratio, x: concentration of analyte (㎍/㎖).

Table 2. Variation in the concentration (㎎/100 g) of steroids **1,2** and triterpenes **5-8** in the *Orostachys japonicus* A. Berger according to the cultivation

	Standard Material (㎎/100 g[a])						
sample	**1**	**2**	**5a**	**5b**	**6**	**7**	**8**
Night-break[b]							
7/2	1.08±0.06	38.71±0.40	7.19±0.04	1.66±0.04	6.69±0.22	75.14±1.44	7.13±0.12
8/25	0.68±0.03	25.20±0.23	6.51±0.07	1.29±0.02	4.92±0.19	53.72±1.43	5.21±0.06
day-length[c]							
13 : 11	0.76±0.02	39.16±0.51	6.69±0.14	2.18±0.09	6.43±0.21	67.97±1.00	6.28±0.06
16 : 08	0.43±0.02	20.11±0.28	4.56±0.16	1.38±0.06	3.44±0.10	38.36±0.60	3.47±0.08
Brightness[d]							
0%e	1.82±0.05	28.13±1.07	7.43±0.06	1.31±0.01	3.06±0.05	40.61±0.51	5.30±0.11
35%	1.78±0.06	39.58±1.45	8.95±0.48	1.67±0.06	4.37±0.15	61.27±2.63	5.87±0.12
55%	2.16±0.07	41.87±2.04	8.90±0.26	1.68±0.04	4.37±0.21	65.04±3.20	6.21±0.11
Nitrogen Fertilizer							
N5[e]	1.23±0.04	27.13±0.87	7.93±0.47	1.67±0.06	6.20±0.26	66.07±1.63	6.87±0.15
N10[e]	1.51±0.05	31.77±1.46	9.26±0.12	2.33±0.06	8.20±0.26	89.93±3.33	9.23±0.25
N20[e]	0.64±0.02	22.63±0.60	4.90±0.44	1.27±0.06	5.03±0.21	49.90±0.56	5.30±0.10
Phosphorus Fertilizer							
P5[e]	0.82±0.03	22.21±0.76	4.44±0.08	1.37±0.06	4.74±0.07	54.73±2.35	5.43±0.25
P10[e]	1.50±0.03	21.70±0.92	6.39±0.11	1.27±0.06	5.17±0.12	50.43±1.96	6.00±0.20
P20[e]	1.07±0.02	26.73±1.25	7.42±0.33	1.84±0.06	6.23±0.29	70.57±2.97	7.27±0.15
Potassium Fertilizer							
K5[e]	2.72±0.11	40.10±1.97	10.47±0.42	2.73±0.06	9.30±0.35	109.87±4.50	11.17±0.21
K10[e]	1.54±0.08	21.33±1.05	6.55±0.23	1.55±0.06	4.67±0.21	56.73±2.34	5.80±0.17
K20[e]	1.31±0.06	87.10±1.21	7.39±0.19	1.77±0.06	6.86±0.14	79.20±2.36	8.11±0.21
Wild							
Before Blooming	1.25±0.06	23.17±0.30	7.43±0.06	1.31±0.01	3.86±0.05	40.61±0.51	5.30±0.11
After Blooming	1.27±0.05	40.60±1.75	6.49±0.26	1.57±0.06	3.63±0.06	53.80±2.00	5.22±0.02

[a]: weight of freeze-drying, [b]: Experimental Day On, [c]: Day/Night, [d]: Light Shielding, [e]: Fertilization (㎏/㏊), **1**: Campesterol, **2**: β-Sitosterol, **5a**: Taraxerone, **5b**: Taraxerone, **6**: β-Amyrin, **7**: (–)-Friedelin, **8**: Glutinol.

brightness control (0%) showed the lowest content of **6**, the lowest contents of **7** and **8** were found on the day-length control 16:08. In fertilization experiments, the highest contents of **5-8** were observed from K5. While the lowest contents of **5** and **6** resulted from P5 and K10, respectively, and those of **7** and **8** from N20. These experiments showed that there is an clear linear relationship between the content of the triterpenes **5-8** and amount of added light. Light would be somewhat unfavorable or in effective to the production of the triterpenes **5-8**. A variety and amount of fertilization may not be concerned to their biosyntheses.

CONCLUSION

A light would be somewhat unfavorable or in effective to the population of the steroids **1** and **2**, and the triterpenes **5-8**. It is suggested that a variety and amount fertilization may not effect to their bio-production. The present investigation disclosed that there are no significant difference in their contents between the cultivated *Orostachys japonicus* A. Berger and wild one. From viewpoint of the content of the steroids **1** and **2**, and the triterpenes **5-8** the quality of cultivated *Orostachys japonicus* A. Berger is not inferior to the wild one.

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