

## Seasonal Changes of Oxindole Alkaloid Contents in the Stem Segments of *Uncaria rhynchophylla*<sup>1)</sup>

SADAHIRO KAWAZOE, CHIZUKO YAMAGUCHI\*, HAJIME MIZUKAMI\*  
and HIROMU OHASHI\*

(Received July 20, 1993)

### Abstract

The seasonal changes of oxindole alkaloid content in stem segments of *Uncaria rhynchophylla* were investigated between July 1986 and June 1987. The highest oxindole alkaloid content in the dried stem segments was observed in July 1986, and the content decreased until the next spring, when it started to increase again. In contrast, high biomass production was obtained in winter (November and December). The optimum season to prepare the crude drug from *U. rhynchophylla* is discussed in relation to oxindole alkaloid content and biomass yield.

### Key words

*Uncaria rhynchophylla*, Rubiaceae, seasonal change, oxindole alkaloid content.

### Introduction

Dried stem segments with hook of *Uncaria rhynchophylla* (MIQ.) MIQUEL (Rubiaceae) have been used as a crude drug called "Cho-to-ko" in Japanese ("Gouteng" in Chinese) in Chinese (Kampo) medicine<sup>2)</sup>. Four oxindole alkaloids (isocorynoxine, isorhynchophylline, corynoxine, and rhynchophylline) isolated from the stem segments of this plant have been reported to reveal various pharmacological activities such as central depressive, antiarrhythmic, hypotensive, and Ca<sup>2+</sup>-channel-blocking actions<sup>3,4)</sup>.

The optimum seasons for harvesting the stem segments from *U. rhynchophylla* describe so far in a number of Chinese or Japanese literatures are inconsistent ranging from early spring to mid winter<sup>5-10)</sup>. Therefore, as a part of our efforts to establish a cultivation method of this plant for domestic production of the crude drug "Cho-to-ko", we examined seasonal changes of the oxindole alkaloid content in the stem segments of *U. rhynchophylla*. This will provide us one of the basic information to elucidate the optimum season for the harvest.

### Materials and Methods

#### *Plant material and cultivation*

*U. rhynchophylla* plants collected locally were transplanted and cultivated at the Medicinal Plant Garden, Nagasaki University under the shade (40% shading rate) for three years before they were used for the experiment. The aerial portions of the plants were harvested every year. The plant had

---

Laboratory of Environmental Health and Toxicology, Department of Food Sciences and Nutrition, Kyoto Prefectural University, Shimogamo, Kyoto 606, Japan

\* Department of Medicinal Plant Research, Faculty of Pharmaceutical Sciences, Nagasaki University, Bunkyo-machi 1-14, Nagasaki 852, Japan

14.  $7 \pm 2.5$  (average  $\pm 95\%$  confidence limit from nine samples) stems in July of 1986, when the experiment started. Two to five stems (at the earlier stages of the experiment) or one stem (at the later stages) was collected once every month from the same plant by cutting at 50cm above the soil. The samples were collected from nine plants each time. After the leaves were removed, the stems were cut at the center of the internodes in order to obtain stem segments with hook, dried at  $60^\circ\text{C}$  for a day and stored in a desiccator.

#### Quantitative analysis of alkaloids

Dried powder of each sample (1g) was reflexed twice for 2 hours each in a mixture of benzene (50ml) and ammonia (3ml). The alkaloid content in the extract was determined by HPLC according to the method described by Yamanaka *et al.*<sup>10)</sup>.

### Results and discussion

The plants showed only vegetative growth without flowering during the cultivation period from July 1986 to June 1987 and the leaves did not fall through the winter. As shown in Fig. 1, biomass yield (dry weight of a stem) from the plant increased through the autumn and reached a maximum in November and December. The biomass yield then decreased until the next May, when it started to increase again.

The seasonal changes of the oxindole alkaloid contents were shown in Fig. 2. The alkaloid con-

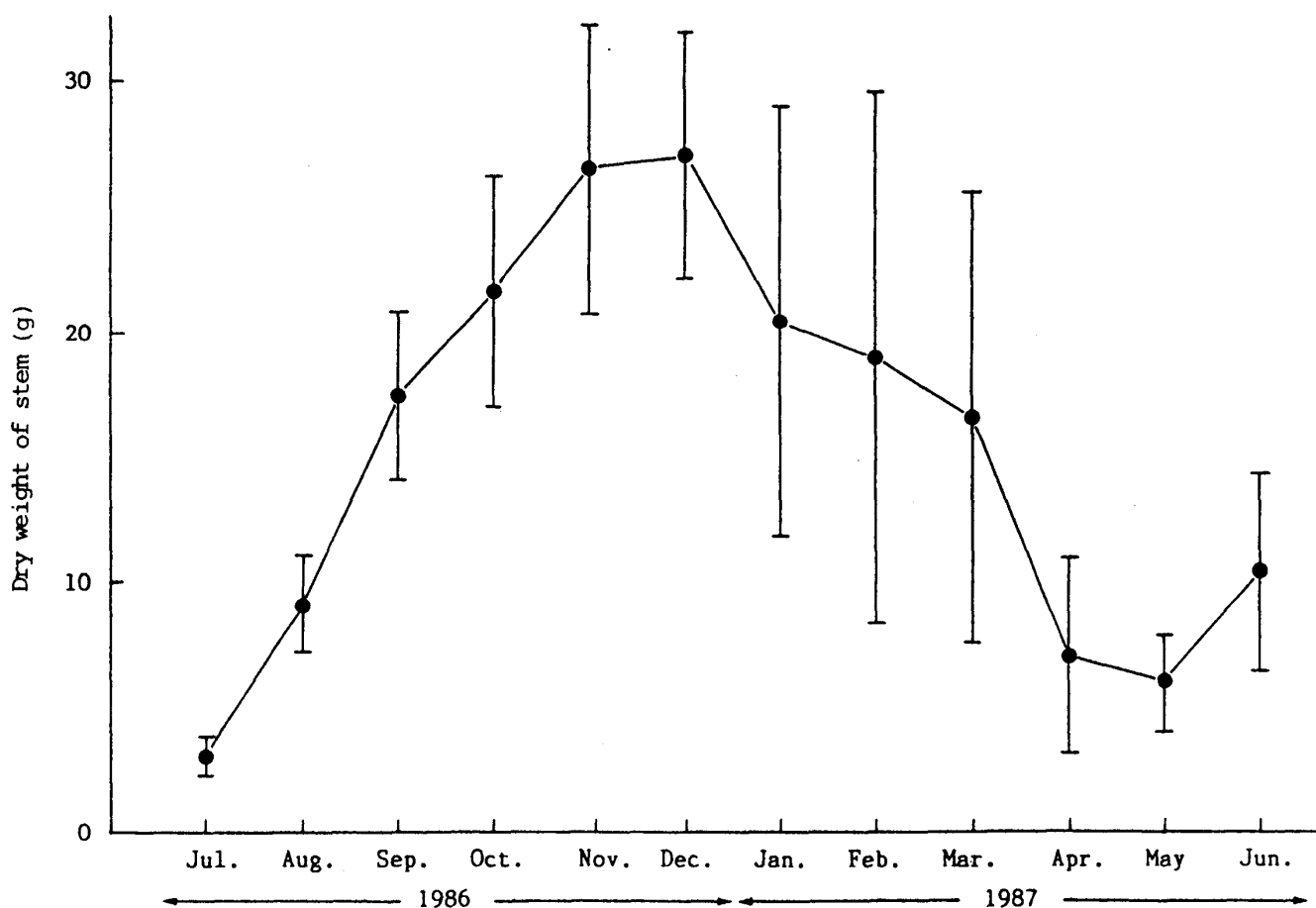


Fig.1. Seasonal change of dry weight of stem harvested from *Uncaria rhynchophylla*.

Each point represents an average with a 95% confidence limit calculated from 9 replicate samples.

tents were highest in July 1986, when the experiment started, and decreased through winter to next spring, when the accumulation started to increase again. No significant difference in the patterns of seasonal changes was observed among four individual oxindole alkaloids (isocorynoxine, isorhynchophylline, corynoxine, and rhynchophylline). Isocorynoxine content was always highest and rhynchophylline content lowest regardless of the time of harvest.

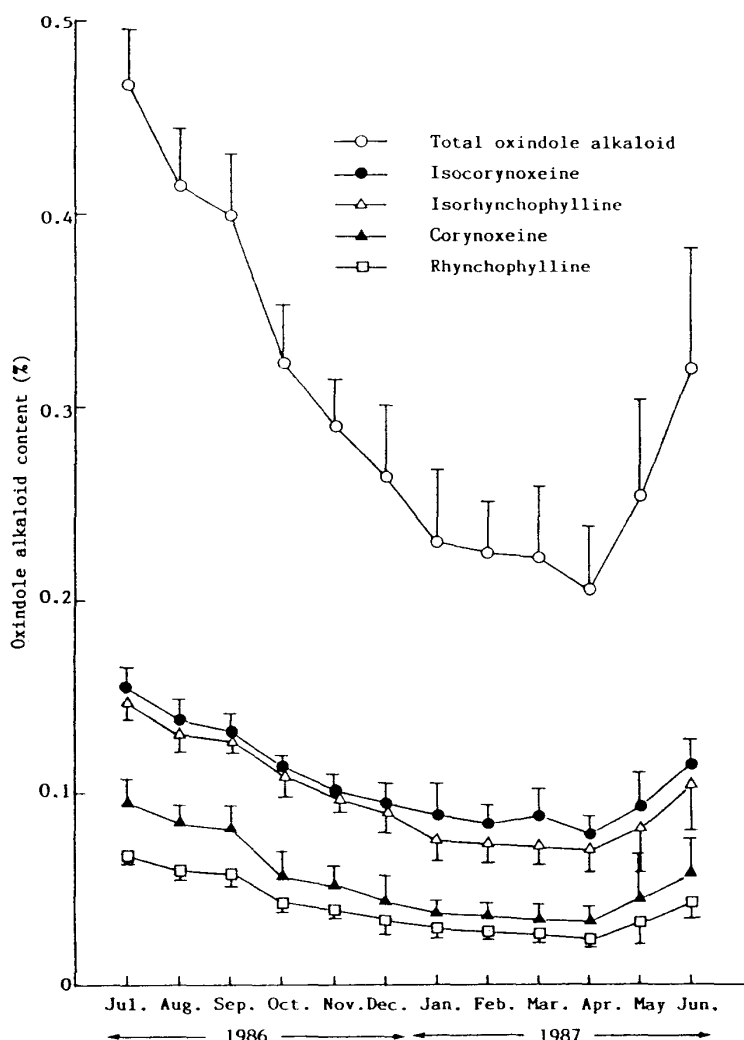


Fig.2. Seasonal changes of oxindole alkaloid contents in the stem segments of *Uncaria rhynchophylla*. Each point represents an average with a 95% confidence limit calculated from 9 replicate samples.

Although the oxindole alkaloid content was low in December ( $0.264 \pm 0.038\%$ ,  $n=9$ ), it was still about 10 to 40-fold higher than the content of the commercially obtained crude drugs imported from China ( $0.006$  to  $0.024\%$ )<sup>22</sup>. It is concluded, therefore, that it is better to harvest the stem segments in November or December when the biomass yield reaches the maximum as far as the plants are cultivated in Japan.

#### Acknowledgements

We wish to thank Mr. K. Yuda and Mr. M. Tagami, the Medicinal Plant Garden of Nagasaki University, for help in the cultivation of *Uncaria rhynchophylla* plants.

### References

- 1) Cultivation and Breeding of *Uncaria rhynchophylla* (MIQ.) MIQUEL: Part X. For part IX in this series, see Kawazoe, S., Mizukami, H., Ohashi, H. (1993) *Shoyakugaku Zasshi* **47**, 316-320.
- 2) Numba, T. (1980) in: *Genshoku Wakanyaku Zukan*, Vol. 2, p.186-188, Hoikusha Publishing Co., Osaka, Japan.
- 3) Ozaki, Y., Harada, M., Sakai, S. (1980) *Japan. J. Pharmacol.* **30**, Suppl. 137.
- 4) Yamahara, S., Miki, S., Matsuda, H. (1987) *Nippon Yakurigaku Zasshi* **90**, 133-139.
- 5) Jiangsu Xin Yixueyuan (1977) in: *Zhongyao Dacidian*, Vol. 2, p.1668-1670, Shanghai Kexue Jishu Chubanshe, Shanghai, China.
- 6) Kimura, K., Suzuki, S. (1979) in: *Shinchu Koutei Kokuyaku Honzo Komoku*, Vol.6, p.368-371, Shyunyodo Shyoten, Tokyo, Japan.
- 7) Sichuan Zhongyaozhi Xiezu Bianxiezhu (1980) in: *Sichuan Zhongyaozhi*, Vol.1, p.197-199, Sichuan Renmin Chubanshe, Chengdu, China.
- 8) Zhejiang Yaoyong Zhiwuzhi Bianxiezhu (1980) in: *Zhejiang Yaoyong Zhiwuzhi*, Part 2, p.1230-1232, Zhejiang Kexue Jishu Chubanshe, Hangzhou, China.
- 9) Fujian Zhongyi Yanjiusuo (1983) in: *Fujian Yaowozhi*, Vol.2, p.324-325, Fujian Kexue Jishu Chubanshe, Fuzhou, China.
- 10) Zhonghua Renmin Gongheguo Weishengbu Yaodian Weiyuanhui (1985) in: *Zhonghuarenmin Gongheguo Yaodian*, Vol.1, p.220, Renmin Weisheng Chubanshe Huaxue Gongye Chubanshe, Beijing, China.
- 11) Yamanaka, E., Kimizuka, Y., Aimi, N., Sakai, S., Haginiwa, J. (1983) *Yakugaku Zasshi* **103**, 1028-1033.
- 12) Kawazoe, S., Mizukami, H., Ohashi, H. (1989) *J. Pharmaco-bio Dyn.* **13**, s-44.