Natural Medicines **51**(5), 417-419 (1997)

Atractylon Is a Major Sesquiterpene in Young *Atractylodes lancea* Plants Raised from Shoot Cultures

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(Received December 19, 1996)

In the rhizomes of young *Atractylodes lancea* plants micropropagated from shoot tips, the major sesquiterpene of the essential oils was atractylon, which is the major sesquiterpene of *A. japonica* or *A. ovata,* though in the rhizomes of older plants, hinesol and β-eudesmol was the major sesquiterpenes. Thus, the accumulation pattern of essential oils varies depending on the plant age. The presence of atractylon in *A. lancea* does not necessarily mean that the plant is a hybrid between *A. lancea* and *A. japonica* or *A. ovata,* both of which contain atractylon as the major sesquiterpene in the rhizome. *A. lancea* plants have an ability to biosynthesize atractylon genetically. The rhizomes of young micropropagated *A. ovata* plants accumulated atractylon as a major essential oil component, but its content was lower than that of the mother plant.

Keywords——*Atractylodes lancea*; Compositae; micropropagation; sesquiterpene; atractylon; essential oil; hybridization

"Jutsu" comprises two kinds of crude drugs, "Byaku-jutsu" and "So-jutsu." The former is prepared from rhizomes of *Atractylodes japonica* KOIDZ. ex KITAM. and *A. ovata* DC. (*A. macrocephala* KOIDZ.) of the family Compositae, and the latter from those of *A. lancea* DC. and *A. chinensis* KOIDZ.[1] These crude drugs are rich in essential oils consisting mainly of sesquiterpenes and polyacetylenic compounds. The essential oil of So-jutsu is characterized by the dominancy of two sesquiterpenes, hinesol and/or β-eudesmol, and a polyacetylenic compound, atractylodin, whereas, that of Byaku-jutsu contains a sesquiterpene, atractylon, as the sole major essential oil component.[2]

The purity test of So-jutsu adopted by the Japanese Pharmacopoeia[1] is to detect atractylon and related compounds by vanillin/concentrated hydrochloric acid method. Because atractylon has been considered characteristic of Byaku-jutsu, and the occurrence of atractylon in essential oils of So-jutsu has been regarded as an evidence of hybridization between the two groups of plants.[3] However, recent extensive surveys of essential oil compositions in the rhizomes of *A. lancea* plants growing wild in China revealed that a group of *A. lancea* plants accumulates atractylon and atractylodin as the major essential oil components.[4] Similar results have been reported by Kawanishi *et al.*[5] and Kohda *et al.*[6] Furthermore, we recently found by restriction fragment length polymorphisms of rDNA,[7] that the presence of atractylon in the rhizome of *A. lancea* is not necessarily an evidence of hybridization of the plant with *A. japonica* or *A. ovata.*

Previously, we micropropagated *A. lancea*[8] and evaluated the clonal plants cultivated in the field for 4 to 5 years.[9] In the present study, we determined the amounts of the essential oil components of *A. lancea* in various developmental stages raised from cultured shoots. In the course of the study, we found that atractylon is the major essential oil component in the rhizome of *A. lancea* plants in their early growth stages though it is a minor essential oil component of the mother plant. The occurrence of atractylon in *A. lancea* rhizome will be discussed in relation to hybridization of the plants with original plants of Byaku-jutsu.

MATERIALS AND METHODS

Shoot culture and field cultivation The plant materials of *A. lancea* were supplied by Hamochi-machi, Sado Island and those of *A. ovata* by Professor T. Kimura, Daiichi College of Pharmaceutical Sciences. The clonal plants were obtained from shoot tips of *A. lancea* (AtT and AtB strains) by the methods described earlier.[8] The AtT plant is essential-oil poor and atractylon-rich, whereas the AtB plant is essential-oil rich and atractylon-poor.[10] The clonal plants of *A. ovata* were raised by the same method as that used for *A. lancea* except that the plant hormones in the shoot multiplication medium used for *A. ovata* were 10^{-6} M indole-3-acetic acid and 10^{-5} M benzyladenine according to the data reported by Hatano *et al.*[11] Fifty *in vitro*

shoots and 50 plantlets were harvested after 30 days of incubation. The potted plantlets were cultivated in a greenhouse for up to 150 days and harvested after 4 months (AtT strain) or after the cultivation periods indicated in Fig. 2 (AtB strain) and in TABLE I (AtO strain). Ten plants of each strain were harvested for the chemical analysis of essential oils. The potted plants of AtT, AtB and AtO strains were transplanted to an experimental field of the College in Niigata City on April 26, June 13 and May 25, respectively, and 10 plants of AtT and AtB and 5 plants of AtO were harvested on the 25th day of each month as shown in Fig. 1, Fig. 2 and TABLE I and used for chemical analysis of essential oils.

Analysis of essential oil components Atractylon in the rhizome of clonally propagated and filed-cultivated plants (AtT strain) was identified by GC-MS. The atractylon, hinesol, β-eudesmol and atractylodin contents were determined by the method described previously.[12]

RESULTS AND DISCUSSION

The shoot cultures and the aerial parts of *in vitro* plantlets of both AtT and AtB strains accumulated no detectable amounts of essential oils. The roots of the *in vitro* plantlets contained a small amount of atractylodin and a trace amount of atractylon. Figure 1 shows changes in the compositions of essential oils in the rhizomes and roots of potted and field-grown plants of AtT strain. The rhizomes of the potted plants accumulated atractylon which was the first major sesquiterpene found in the rhizome during the developmental stages starting from cultured shoots. A small amount of β-eudesmol was detected in the rhizome for the first time when the plants were cultivated in the field for two months. The accumulation of hinesol did not occur till autumn or 6 months after transplantation of the plants to the field. The total amount of sesquiterpenes decreased during winter season and gradually increased from spring to autumn of the next cultivation year. It is noteworthy that atractylon is the dominant constituent in essential oils of the rhizomes of young plants derived from shoot cultures. On the other hand, atractylodin was detected in the rhizome at any growth stage including potted plantlet rhizomes. It is evident that the essential oil profile of *A. lancea* varies considerably as the plant grow, and the 4- or 5-year-old plants derived from shoot cultures had an essential oil composition similar to that of the control plants propagated by the division of rhizomes.[9]

Figure 1 also shows the analytical data on the essential oil components in the root of young plants derived from shoot cultures of AtT. Atractylon was always detected in small amounts in the root cultivated in pots or in the field together with variable amounts of atractylodin. Eudesmol was detected in root samples

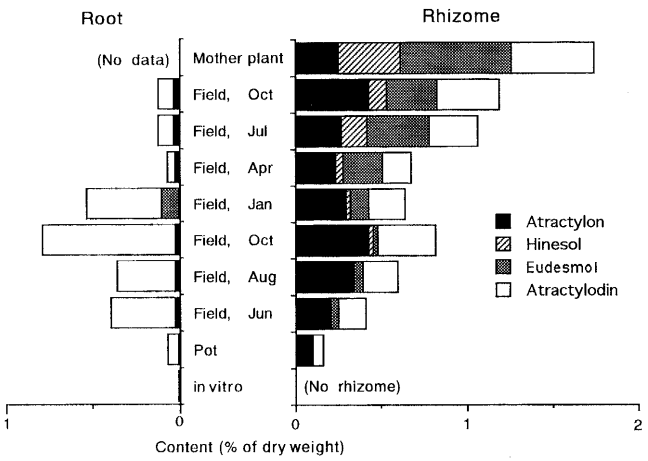

Fig. 1. Changes in the Essential Oil Compositions in the Underground Parts of Potted and Field-Grown *Atractylodes lancea* Plants (AtT, Essential Oil-Poor and Atractylon-Rich Strain) Derived from Shoot Tip Cultures

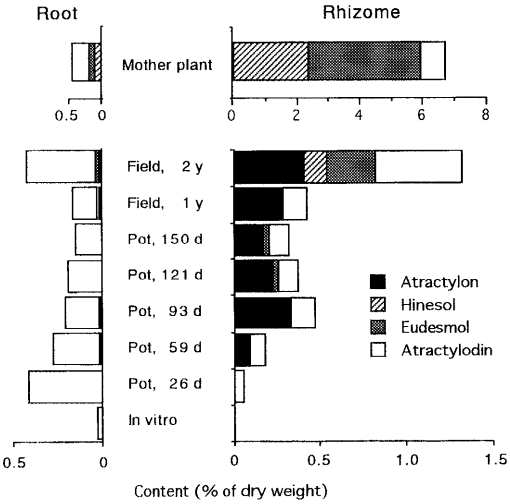

Fig. 2. Changes in the Essential Oil Compositions in the Underground Parts of *in vitro*, Potted and Field-Grown *Atractylodes lancea* Plants (AtB, Essential Oil-Rich and Atractylon-Poor Strain) Derived from Shoot Tip Cultures

harvested in January. No hinesol was found in roots throughout the cultivation period of up to two years. Essential oils were hardly detected in the aerial parts of *A. lancea* plants of both strains in pot or field conditions as observed in our previous study.[12]

To find out whether the dominancy of atractylon in the rhizome of young plants of *A. lancea* is specific to atractylon-rich strains such as AtT, which is positive to the purity test of So-jutsu in the Japanese Pharmacopoeia, we carried out an analogous experiment with an atractylon-poor strain, AtB, which contained more than 6% of essential oils on a dry weight basis but scarcely any atractylon in the rhizome,[10] as shown in Fig. 2. Atractylon was again the major sesquiterpene in the

TABLE I. Change in the Atractylon Content in the Rhizomes and the Roots of Potted Plants and Field-Grown Plants Derived from Shoot Tip Cultures of *Atractylodes ovata*

Plant materials	Month of harvest	Atractylon content (%) in	
		Rhizome	Root
Potted plants[a)]	August	nd	tr
	September	tr	tr
	October	0.12	0.02
	November	nd	tr
	April	0.07	0.01
Field-grown plants[b)]	August	0.27	tr
	November	0.27	0.11

a) *In vitro* plantlets were potted on July 31. Ten plants were harvested at the end of the months indicated.
b) Potted plants were transplanted to the experimental field on May 25. Five plants were harvested on August 25 and November 25.
nd : not detected. tr : trace (<0.05%).

essential oil of the rhizome and root of young plants of AtB strain up to 2-year-old. These findings show that atractylon is a dominant component in the essential oils of young *A. lancea* plants both of atractylon-rich and atractylon-poor strains and suggest that atractylon is not an extraneous sesquiterpene brought into *A. lancea* by hybridization with *A. ovata* or *A. japonica* but an innate constituent of *A. lancea*.

TABLE I shows the atractylon contents in the underground parts of young plants derived from shoot cultures of *A. ovata* (AtO strain). The mother plant contained 1.21% atractylon in the rhizome as the sole major sesquiterpene constituent and not a detectable amount of hinesol, eudesmol or atractylodin.[7] Unlike *A. lancea*, the potted plants accumulated atractylon in the rhizome and root in October and in April of the next year. The rhizomes of the plants grown in the field for several months accumulated atractylon, though its content (0.27%) was lower than that of the mother plant.

The present results demonstrated that atractylon is the first major sesquiterpene to be accumulated in the underground parts of young plants of *A. lancea* and *A. ovata*, indicating that atractylon is also an inherent constituent of *A. lancea*. Since this compound is also a major sesquiterpene in *A. chinensis* KOIDZ. and *A. koreana* KITAM. (*A. lancea* DC. var. *simplicifolia* KITAM.),[4c)] it seems that atractylon is an essential oil component common to the genus *Atractylodes*, and that, accordingly, atractylon-rich and -poor plants of *A. lancea* are to be considered as chemical variants within the species as proposed by Takeda *et al.*[4c)] The annual and seasonal changes in essential oil compositions and their contents of *A. lancea* will be reported separately.

Acknowledgements: The authors thank Prof. Takeatsu Kimura, Daiichi College of Pharmaceutical Sciences and Hamochi-machi Town Office for supplying the plant materials.

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