On the Volatile Components of *Heterotropa takaoi* and Related Species (Aristolochiaceae)

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The volatile components of the leaves of four species and five varieties of the section Bicornes of *Heterotropa* were examined by gas chromatography and gas chromatograph-mass spectrometry. The analytical results showed that the species of the section contain phenylpropanes, terpenes, and a paraffin as main components. The components have been used to construct phylogenetic trees of the section Bicornes. Despite some discrepancies, the phylogenetic tree obtained for the section seems to be relevant at the tribal revel. Among the species of the section, geographical variation of twelve localities of *H. takaoi* also were examined and three chemical types (myristicin, safrole, *n*-pentane-elemicin types) of *H. takaoi* are distinguished on the basis of their components.

Introduction

The genus *Asarum* possessing about 70 species has a wide distribution in Asia, Europe, and North America. All the species of the genus *Asarum* show similar morphological characters. According to Engler's classification [1], all species are placed in one genus *Asarum*. In contrast, the genus *Asarum* is divided into four genera *(Asarum, Heterotropa, Asiasarum, Hexastylis)* in Maekawa's systems [2]. In this work, Maekawa's classification was used. Although it is difficult to distinguish each species of *Heterotropa* in Japan and Formosa on morphological data, Maekawa reported about 50 species belonging to the seven sections (Euheterotropa, Bicornes, Ranyo, Sakawanae, Asiasarum, Annularia, Kanaoi) for this group.

From the viewpoint of karyology, Ono [3] examined 22 species of *Heterotropa* ($2n = 24$) together with three species of *Hexastylis* ($2n = 26$) from North America, one species of *Asarum* ($2n = 26$) from Canada, and one species of *Asiasarum* ($2n = 26$) from Japan. As for polyploidy, two species were found in *Heterotropa; H. megacalyx* ($2n = 48$), *H. takaoi* var. *rigescens* ($2n = 48$). In *H. takaoi* var. *regescens,* diploid ($2n = 24$) were observed together with the tetraploid ones ($2n = 48$), even in the same individual. Recently Yuasa and Maekawa [4] examined chromosomes of 14 species and 3 varieties of *Heterotropa* collected in southern part of Japan (Osumi, Amami, Okinawa, Yaeyama islands). The *Heterotropa* species examined all exhibited a chromosome number of $2n = 24$. In these species the reduction of chromosome number from $2n = 26$ to $2n = 24$ may begin with two chromosomes of the terminal type.

A phytochemical study of *Heterotropa* was first done by Nagasawa [5]. He analyzed the essential oils of eleven species by infrared spectroscopy and suggested the presence of safrole, elemicin, and limonene in the essential oils. Saiki [6] reported gas chromatography (GLC) and gas chromatograph-mass spectrometry (GC-MS) of phenol ether and terpene components of 34 species of *Heterotropa*. Biering [7] and Endo [8] reported geographical variation of *Asarum europaeum* and *Asarum caulescens* based on their phenolethers and terpenes. In the course of phytochemical study of *Heterotropa,* we have investigated the components of 46 species by means of GC-MS [9]. In order to throw more light on chemical variation in *Heterotropa,* chemical survey of volatile components of *Heterotropa takaoi* and related species collected from various locations in Japan, were carried out. On the basis of the results obtained, some possible relationships between botanical classification of the species and chemical similarities of the composition of their volatile oils are discussed.

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Experimental

Plant materials

The following four species and five varieties belonging to the section Bicornes in *Heterotropa* have been investigated in this work:

1. *Heterotropa takaoi* F. Maekawa (12 locations), *H. takaoi* var. *hisauchii* F. Maekawa (3 locations), *H. takaoi* var. *elongata* F. Maekawa, *H. takaoi* var. *kurosawae* F. Maekawa. 2. *H. yoshikawai* F. Maekawa. 3. *H. megacalyx* F. Maekawa. 4. *H. fauriei* F. Maekawa, *H. fauriei* var. *nakaii* F. Maekawa, *H. fauriei* var. *serpens* F. Maekawa. Voucher specimens are available for inspection at Herbarium of Faculty of Integrated Arts and Sciences, Hiroshima University.

Isolation and identification of the components of the ether extracts

The fresh whole plants (10–250 g) were collected during flowering seasons. The plants which divided into leaves and roots were chopped up, then, the cut materials were extracted with diethyl ether.

In the case of the study of distribution of volatile components of some organ of *H. takaoi,* individual organs were chopped up to use for analysis. The oily matter which obtained by evaporation of the solvent was analyzed by GLC and GC-MS comparing with the spectra of authentic specimens. The contents of the volatile oils of the fresh leaves and roots were 0.1% and 0.2% of the fresh weight, respectively.

Quantitative GLC analysis was carried out on a gas chromatograph GC-9A (Shimadzu Co. Ltd., Japan) which was combined with integrator and equipped with a 0.25 mm × 50 m glass capillary column coated with OV-101. The column temperature was programmed from 100 to 200 °C (2 °C/min). GC-MS was measured with JMS-D100 mass spectrometer coupled with JGC-20KP gas chromatograph equipped with a 1 m × 3 mm glass column containing 3% OV-101 on 100–200 mesh Chromosorb WAW. Helium was used as the carrier gas at flow rate of 15 ml/min. The column temperature was programmed from 50 to 200 °C at a rate of 5 °C/min.

Statistical analyses of the components of the leaves of the species

All analyses were done using a NEC PC-9801 personal computer equipped with multivariate analysis program soft by Microsystems Co. Ltd. We used a cluster (dendrogram) analysis in order to examine differences among or inter species. This distance measure used was 1-γ (γ = Peason correlation coefficient) and centroid (average) clustering.

Results and Discussion

Analytical data

The analytical results of the ether extracts of the leaves and roots of four species and five varieties belonging to the section Bicornes examined in this study were as follows:

Heterotropa takaoi F. Maekawa (collected from Kyoto, food plant of *Luehdorfia japonica* Leeck.)

Leaves and petioles: α-pinene (0.6%), camphene (0.1%), β-pinene (3.2%), *p*-cymene (1.1%), myrcene (1.6%), limonene (0.3%), 1,8-cineole (0.4%), linalool (1.2%), safrole (4.4%), β-farnesene (3.5%), myristicin (46.7%), *n*-pentadecane (13.6%), elemicin (22.4%), 1-allyl-2,3,4,5-tetramethoxybenzene (ATMB) (0.4%).

Roots: α-pinene (17.9%), camphene (10.2%), β-pinene (16.2%), *p*-cymene (0.8%), *trans*-β-ocimene (0.6%), limonene (5.1%), borneol (6.1%), safrole (0.4%), methyleugenol (3.3%), γ-elemene (0.7%), myrisiticin (2.5%), *n*-pentadecane (2.6%), elemicin (28.0%), 1-allyl-2,3,4,5-tetramethoxybenzene (ATMB) (0.4%).

Heterotropa takaoi var. *hisauchii* F. Maekawa
(a) (collected from Nagano Prefecture)

Leaves and petioles: α-pinene (2.3%), camphene (1.0%), β-pinene (12.3%), *p*-cymene (0.7%), myrcene (1.4%), limonene (0.6%), 1,8-cineole (0.7%), linalool (0.9%), safrole (52.4%), β-farnesene (0.9%), α-bergamotene (0.6%), caryophyllene (0.7%), myristicin (0.4%), *n*-pentadecane (16.3%), elemicin (8.2%), unknown (3.2%).

Roots: α-pinene (7.9%), camphene (3.5%), β-pinene (11.3%), *p*-cymene (0.1%), *trans*-β-ocimene (0.7%), limonene (1.7%), borneol (3.0%), methyleugenol (0.8%), *n*-pentadecane (4.7%), elemicin (61.2%), ATMB (0.6%), unknown (4.4%).

(b) (collected from Wakayama)

Leaves and petioles: α-pinene (0.6%), camphene (0.3%), β-pinene (2.7%), *p*-cymene (0.1%), myrcene (0.8%), limonene (0.4%), 1,8-cineole (0.5%), linalool (0.2%), safrole (60.7%), β-farnesene (1.5%), caryophyllene (0.6%), myristicin (4.2%), *n*-pentadecane (25.8%), elemicin (1.0%), unknown (0.6%).

Roots: α-pinene (10.7%), camphene (6.5%), β-pinene (9.3%), *p*-cymene (0.2%), *trans*-β-ocimene (2.1%), limonene (2.1%), borneol (4.5%), safrole (3.2%), methyleugenol (5.8%), γ-elemene (0.3%), *n*-pentadecane (2.7%), elemicin (48.8%), ATMB (2.7%), unknown (3.3%).

(c) (collected from Gifu Prefecture)

Leaves and petioles: α-pinene (3.3%), camphene (1.4%), β-pinene (6.9%), *p*-cymene (0.5%), myrcene (1.0%), limonene (0.8%), 1,8-cineole (1.0%), linalool (0.6%), safrole (57.1%), β-farnesene (0.9%), myristicin (0.2%), *n*-pentadecane (22.6%), elemicin (3.0%), ATMB (0.7%).

Roots: α-pinene (19.2%), camphene (8.9%), β-pinene (20.4%), *p*-cymene (1.9%), limonene (9.5%), borneol (6.4%), safrole (0.7%), methyleugenol (0.5%), γ-elemene (0.2%), *n*-pentadecane (2.0%), elemicin (21.5%), ATMB (1.4%), unknown (7.6%).

Heterotropa takaoi var. *kurosawae* F. Maekawa
(collected from Sidzuoka Prefecture)

Leaves and petioles: myrcene (trace), 1,8-cineole (trace), myristicin (98.2%).

Roots: α-pinene (5.0%), camphene (3.0%), β-pinene (3.3%), 1,8-cineole (1.3%), borneol (4.4%), ATMB (18.5%), safrole (1.0%), *n*-pentadecane (2.0%), elemicin (61.5%).

Heterotropa takaoi var. *elongata* F. Maekawa
(collected from Ishikawa Prefecture)

Leaves and petioles: α-pinene (3.0%), camphene (1.6%), β-pinene (10.3%), *p*-cymene (0.6%), myrcene (1.3%), limonene (1.5%), 1,8-cineole (1.6%), linalool (1.0%), safrole (58.3%), β-farnesene (0.2%), myristicin (0.5%), *n*-pentadecane (14.9%), elemicin (3.0%), unknown (2.2%).

Roots: α-pinene (6.8%), camphene (3.7%), β-pinene (4.9%), *p*-cymene (0.6%), *trans*-β-ocimene (0.5%), limonene (3.8%), borneol (2.6%), safrole (1.5%), methyleugenol (1.2%), γ-elemene (0.3%), myristicin (2.2%), *n*-pentadecane (1.2%), elemicin (37.5%), ATMB (27.7%), unknown (3.7%).

Heterotropa yoshikawai F. Maekawa
(collected from Niigata Prefecture)

Leaves and petioles: α-pinene (4.2%), camphene (1.7%), β-pinene (2.5%), myrcene (3.7%), α-phellandrene (6.0%), *p*-cymene (trace), linalyl acetate (1.5%), borneol (1.2%), bornyl acetate (1.2%) methyleugenol (2.2%), caryophyllene (5.2%), α-cubebene (2.0%), α-humulene (2.5%), myristicin (24.5%), asaricin (11.2%), *n*-pentadecane (11.0%), elemicin (5.7%), asarone (8.7%), ATMB (2.5%), unknown (2.5%).

Roots: *n*-undecane (trace), α-pinene (11.5%), camphene (7.6%), β-pinene (8.9%), myrcene (4.3%), borneol (5.7%), bornyl acetate (7.5%), methyleugenol (2.3%), *n*-pentadecane (4.0%), elemicin (21.4%), ATMB and its isomer (24.7%), unknown (2.1%).

Heterotropa megacalyx F. Maekawa
(collected from Niigata Prefecture)

Leaves and petioles: α-pinene (8.4%), camphene (6.1%), β-pinene (6.9%), myrcene (4.1%), camphor (2.8%), borneol (9.9%), bornyl acetate (1.5%), methyleugenol (4.1%), α-bergamotene (1.6%), γ-elemene (2.9%), *n*-pentadecane (10.7%), elemicin (15.2%), ATMB and its isomers (18.0%), asarone (5.9%), unknown (1.9%).

Roots: *n*-undecane (2.0%), α-pinene (2.7%), camphene (1.0%), β-pinene (5.9%), myrcene (5.6%), 1,8-cineole (11.4%), linalool (1.5%), camphor (0.5%), borneol (1.0%), β-terpineol (1.8%), α-terpineol (3.5%), α-cubebene (1.7%), α-copaene (5.9%), elemene (3.5%), α-bergamotene (1.0%), caryophyllene (0.5%), *n*-pentadecane (trace), E-β-farnesene (1.5%), α-curcumene (0.5%), α-cedrene (0.5%), elemicin (25.0%), ATMB (22.0%), unknown (3.0%).

Heterotropa fauriei F. Maekawa
(collected from Yamagata Prefecture)

Leaves and petioles: α-pinene (6.0%), camphene (2.8%), β-pinene (11.6%), myrcene (2.1%), *p*-cymene (1.3%), limonene (6.7%), 1,8-cineole (1.0%), linalool (1.8%), safrole (0.1%), β-farnesene (3.6%), α-bergamotene (7.1%), caryophyllene (0.4%), myristicin (1.3%), ATMB and its isomers (24.3%).

Heterotropa fauriei var. *nakaii* F. Maekawa
(collected from Nagano Prefecture)

Leaves and petioles: α-pinene (7.6%), camphene (3.4%), β-pinene (17.9%), *p*-cymene (2.6%), myrcene (3.1%), limonene (2.2%), 1,8-cineole (2.5%), linalool (3.6%), safrole (0.2%), β-farnesene (0.6%), α-bergamotene (3.3%), caryophyllene (3.1%), myristicin (6.1%), *n*-pentadecane (41.6%), elemicin (2.2%).

Roots: α-pinene (19.9%), camphene (12.5%), β-pinene (16.3%), *p*-cymene (1.4%), *trans*-β-ocimene (3.4%), limonene (5.0%), borneol (8.1%), *n*-pentadecane (4.4%), elemicin (23.5%), ATMB (1.5%), unknown (4.0%).

Heterotropa fauriei var. *serpens* F. Maekawa
(collected from Nagano Prefecture)

Leaves and petioles: α-pinene (4.0%), camphene (2.4%), β-pinene (4.1%), myrcene (0.7%), limonene (0.6%), 1,8-cineole (0.7%), linalool (0.1%), safrole (0.5%), β-farnesene (1.7%), α-bergamotene (0.2%), myristicin (0.6%), *n*-pentadecane (35.6%), elemicin (0.5%), ATMB (48.5%).

Roots: α-pinene (12.5%), camphene (7.3%), β-pinene (5.7%), *p*-cymene (0.8%), *trans*-β-ocimene (1.0%), limonene (3.0%), borneol (5.4%), *n*-pentadecane (3.8%), elemicin (11.1%), ATMB (48.5%), unknown (0.2%).

The components of the four species and five varieties of the section Bicornes

Components of the species belonging to the section Bicornes (*H. takaoi* F. Maekawa, *H. takaoi* var. *hisauchii, H. takaoi* var. *kurosawae, H. takaoi* var. *elongata, H. yoshikawai, H. megacalyx, H. fauriei* F. Maekawa, *H. fauriei* var. *nakaii, H. fauriei* var. *serpens*) were investigated. The distribution of these species and population samples are shown in Fig. 1. The volatile components were obtained by ether extracts and then analyzed by means of GC and GC-MS. The results showed that all the species tested contained terpenes, phenylpropanes, and a paraffin. We have found that eight monoterpenes, three sesquiterpenes and *n*-pentadecane were identified as the volatile components of the leaves of section Bicornes. Most of the species of section

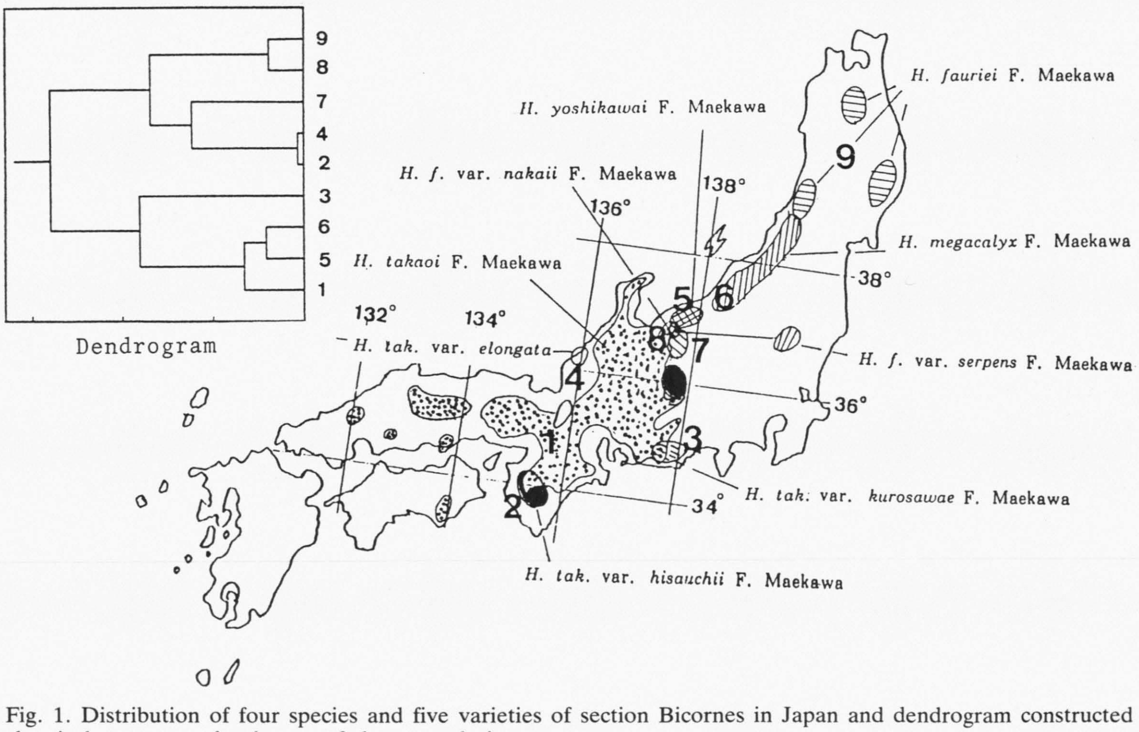

Fig. 1. Distribution of four species and five varieties of section Bicornes in Japan and dendrogram constructed from chemical parameters by the use of cluster analysis.

Bicornes contained phenylpropanes many more than terpenes. The structures of the main components of the volatile oils are shown in Fig. 2. Both species of *H. takaoi* var. *elongata* and *H. takaoi* var. *hisauchii* contained a lot of safrole (58.3%, 52.4%) together with *n*-pentadecane (14.9%, 16.3%) as main components. A large amount of myristicin was obtained from the volatile oils of the species of *H. takaoi* var. *kurosawae* (98.2%) and *H. yoshikawai* (24.5%). The volatile oils of *H. fauriei* var. *elongata* and *H. megacalyx* contained a considerable amount of ATMB (48.5%, 18%). *H. fauriei* var. *nakaii* contained *n*-pentadecane (41.6%) many more than α- and β-pinene (25.5%) in the leaf oils. The unique paraffin profile might be an adaptation to such cold climates.

The dendrogram of the results of cluster analysis of the components of four species and five varieties of the section Bicornes based on the volatile components of the leaves is shown in Fig. 1. The cluster membership of the species is indicated on the map (Fig. 1). From this, the four species and five varieties belonging to the section Bicornes are divided into two groups based on the component similarity. Most of species in group one (1, 3, 5, 6) contained a large amount of myristicin together with small amounts of elemicin and terpenes. The other group (2, 4, 7, 8, 9) contained safrole, *n*-pentadecane and/or ATMB as main components.

We have found that *H. takaoi* (1) is related to both *H. yoshikawai* (5) and *H. megacalyx* (6) chemically. *H. takaoi* var. *kurosawae* (3) also related to the above three species. On the other hand, *H. takaoi* var. *hisautii* (2) is closely related to *H. takaoi* var. *elongata* (4). The remaining three species, *H. fauriei* F. Maekawa (9), *H. fauriei* var. *serpens* (7) and *H. fauriei* var. *nakaii* (8) related to the above two species (2, 4) chemically. The possible interrelationships between chemical and botanical classifications of section Bicornes are shown in Fig. 3. Despite some discrepancies, the phylogenetic tree (dendrogram, Fig. 1) obtained from the components of the species of the section Bicornes seems to be relevant at the tribal level.

The chemical differences among these four species and five varieties were only weakly reflected by morphology. The terpenes and phenylpropanes from various species have been used for successfully in elucidation of genetic relationships within and among species.

Geographical variations of the volatile components of the leaves of Heterotropa takaoi

Geographical variation of twelve localities of *H. takaoi* in Japan were investigated. Eight monoterpenes, three sesquiterpenes, four phenylpropanes and *n*-pentadecane were identified as the volatile components of the leaves of *H. takaoi* F. Maekawa. The distribution of these constituents in the various

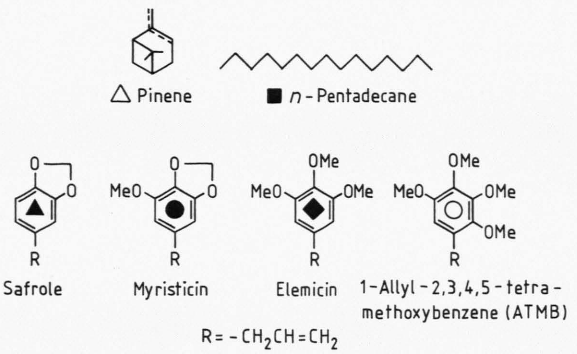

Fig. 2. Structures of the main components of the leaves of section Bicornes.

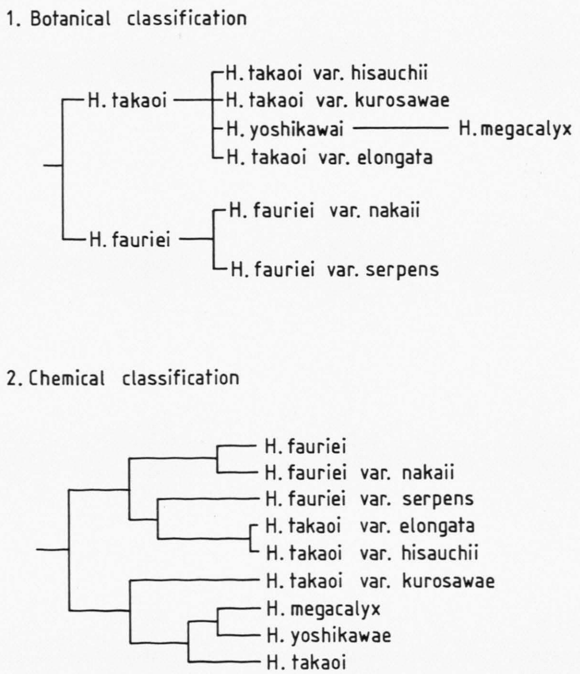

Fig. 3. Interrelationships between chemical and botanical classification of section Bicornes.

populations are shown in Table I. In the volatile oils, myristicin, safrole, *n*-pentadecane, elemicin, ATMB and α- and β-pinenes were the main components. As shown in Table I, the species of all localities contained terpenes, phenylpropanes and *n*-pentadecane with wide range of contents. The distinction of phenylpropane, terpenes and *n*-pentadecane of the plants examined showed a characteristic pattern (Table I). More than 30% of myristicin were present in the plants of 6 localities (code No. 2, 3, 4, 5, 6, 10) in the central Japan. The plants of remaining six localities where are the east and west distincts of *H. takaoi* distribution, however, contained myristicin less than 10% but contained many more safrole (code No. 1, 7, 8), elemicin (code No. 9) and *n*-pentadecane (code No. 11, 12).

The relationships between the chemical components and the population samples of 12 localities are shown in Fig. 4. We can divide the plants of 12 localities into three chemotypes: myristicin type, safrole type and *n*-pentadecane-elemicin type. The myristicin type was wide distributed in central Japan. Safrole type was found in a rather more restricted area as compared with myristicin type. *n*-Pentadecane-elemicin type is distributed in the eastern parts of the myristicin type population. These phytochemical results suggest that myristicin is a characteristic component of *Heterotropa takaoi*.

It may be seen that the species belonging to the safrole type and *n*-pentane-elemicin type may be hybrids between the other species of the section Bicornes (*H. takaoi* var. *hisauchii, H. takaoi* var. *elongata, H. fauriei* var. *nakaii*) on the basis of the components of the species.

Fig. 4 shows the results of cluster analysis based on the components above mentioned. As shown in the Fig. 4, code No. 2, 3, 4, 5, 7, 8 and 10 composed of one group. On the other hand, code No. 1, 6, 9, 11 and 12 differed from the above group.

Components of six organs of H. takaoi

The components of six organs (leaf, root, petiole, calyx, subterranean stem, shoot) of *H. takaoi* distributed in Hiroshima [10] were examined. Ten monoterpenes, four sesquiterpenes, four phenylpropanes and *n*-pentadecane were identified by GC and GC-MS. Table II shows the analytical results of six

Table I. Geographical variations of the volatile components of the leaves of *Heterotropa takaoi*.

Component	Location (code No.) 1	2	3	4	5	6	7	8	9	10	11	12
α-pinene	1.0	0.3	0.4	0.6	0.6	6.1	3.3	1.1	9.3	1.9	7.0	4.1
camphene	0.5	0.2	0.3	0.3	0.1	3.2	1.4	0.5	5.5	1.1	2.8	1.9
β-pinene	4.9	2.6	1.0	3.5	3.2	7.0	6.9	3.7	19.5	10.0	19.7	10.3
p-cymene	1.6	0.2	0.3	–	1.1	3.0	0.5	0.1	4.2	1.1	–	0.6
myrcene	0.7	0.1	0.4	0.1	1.6	4.5	1.0	0.4	1.3	1.6	3.2	0.6
limonene	1.0	0.1	0.2	0.1	0.3	0.1	0.8	0.3	3.6	0.6	0.6	2.5
1,8-cineole	1.5	0.2	0.3	0.1	0.4	1.6	1.0	0.4	4.5	0.8	1.0	1.8
linalool	1.0	0.3	0.3	–	1.2	1.9	0.6	0.8	3.1	0.1	3.8	0.3
safrole	63.4	0.2	1.7	0.7	4.4	–	57.1	47.1	0.1	1.7	–	5.5
β-farnesene	5.7	0.5	0.4	2.2	3.5	3.3	0.9	3.3	0.6	1.4	1.8	2.7
α-bergamotene	3.6	0.2	0.5	–	–	–	–	3.3	–	–	–	7.2
caryophyllene	10.0	1.6	1.3	2.0	–	5.8	–	3.1	–	–	2.3	1.2
myristicin	2.9	68.2	77.8	65.7	46.7	30.7	0.2	7.2	0.7	34.2	–	1.1
n-pentadecane	0.1	23.4	13.4	21.9	13.5	13.0	22.6	23.7	14.1	28.8	44.6	29.8
elemicin	1.0	1.4	0.8	2.5	22.4	4.6	3.0	3.9	33.0	2.4	6.0	15.6
1-allyl-2,3,4,5-tetramethoxy-benzene	0.1	0.3	0.9	0.3	0.8	14.3	0.7	1.1	0.5	14.3	7.2	17.5
terpene	32.5	6.2	5.4	8.9	12.0	36.5	16.4	17.0	51.6	18.6	42.2	36.0
phenylpropane	67.4	70.4	81.2	69.2	74.3	49.6	61.0	59.3	34.3	52.6	13.2	34.2
paraffin	0.1	23.4	13.4	21.9	13.5	13.9	22.6	23.7	14.1	28.8	44.6	29.8

Locations (Prefecture): 1. Hiroshima, 2. Tottori, 3. Okayama, 4. Hyogo, 5. Kyoto, 6. Mie, 7. Gifu, 8. Shizuoka, 9. Toyama, 10. Nagano (Iida), 11. Nagano (Ookuwa), 12. Gifu (Ena).

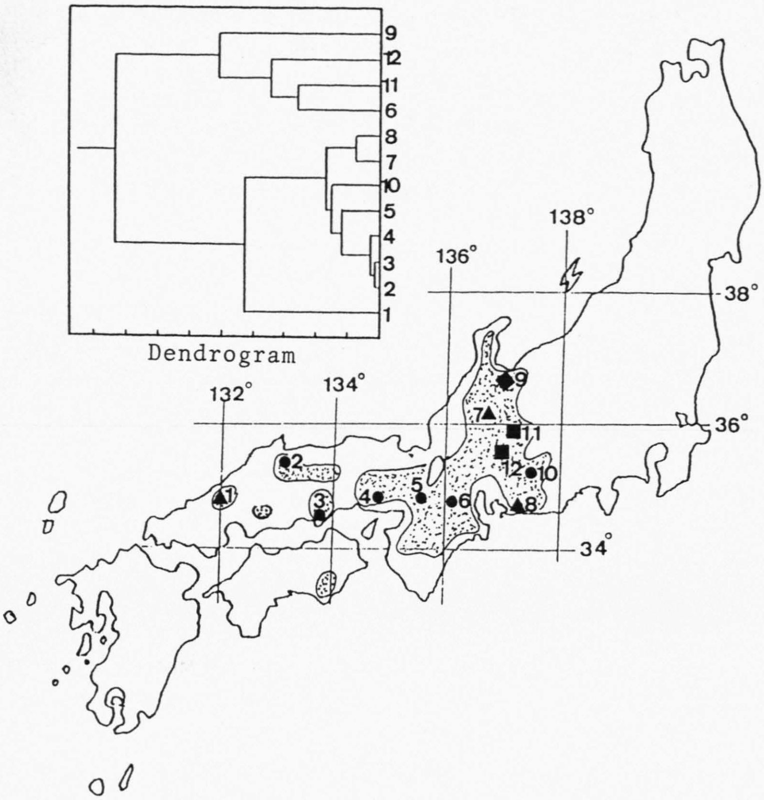

Fig. 4. Distribution of twelve localities of *Heterotropa takaoi* and dendrogram constructed from chemical parameters by the use of cluster analysis.

Table II. Distribution of volatile components of some organs of *Heterotropa takaoi* (from Hiroshima, population No. 1).

Compound	Leaf	Petiole	Subterranean stem	Root	Calyx	Shoot
α-pinene	0.9	5.9	14.1	21.8	2.4	11.0
camphene	0.3	2.8	6.6	9.6	1.3	5.1
β-pinene	6.0	6.1	14.0	22.5	3.6	13.6
p-cymene	0.4	1.3	1.4	2.5	2.8	1.5
trans-β-ocimene	–	–	2.9	4.5	–	–
myrcene	2.0	0.5	–	–	0.6	1.0
limonene	1.3	1.5	2.3	4.0	0.5	2.4
1,8-cineole	1.9	2.5	3.0	4.3	1.0	2.9
linalool	0.8	0.7	–	–	–	1.4
borneol	–	–	5.6	5.6	–	–
safrole	69.3	53.8	0.3	1.0	12.3	22.6
β-farnesene	4.4	6.4	–	–	5.5	6.9
α-bergamotene	3.3	0.5	–	–	0.8	1.0
γ-elemene	–	–	1.9	2.3	–	–
caryophyllene	8.6	4.3	–	–	0.9	0.8
myristicin	0.1	–	–	–	0.3	–
n-pentadecane	0.4	–	0.8	1.3	67.4	–
elemicin	–	13.5	43.6	19.2	0.6	29.1
1-allyl-2,3,4,5-tetra-methoxybenzene	0.3	0.2	3.5	1.4	–	0.7
terpene	20.9	32.5	51.8	77.1	19.4	43.6
phenylpropane	69.7	67.5	49.4	21.6	12.9	52.4
paraffin	0.4	–	0.8	1.3	67.4	–

organs of *H. takaoi*. The components of the leaves contained safrole of 69.3%, while subterranean stems contained elemicin of 43.6%. Roots contained pinene of 44.3% and calyces contained *n*-pentadecane of 67.4%. Both leaves and petioles contained a large amount of safrole. The components of petioles were similar to those of shoots and the components of subterranean stems are also similar to those of roots. The components of leaves and calyces were similar to those of petioles and shoots. Different terpene and phenylpropane profiles might evolve in the different organs due to different biotic selection forces. The dendrogram of the components of some organs by cluster analysis is shown in Fig. 5. The results of cluster analysis (Fig. 4) showed that the components of petioles were closely related to those of shoots and leaves, and the components of subterranean stems related to those of roots.

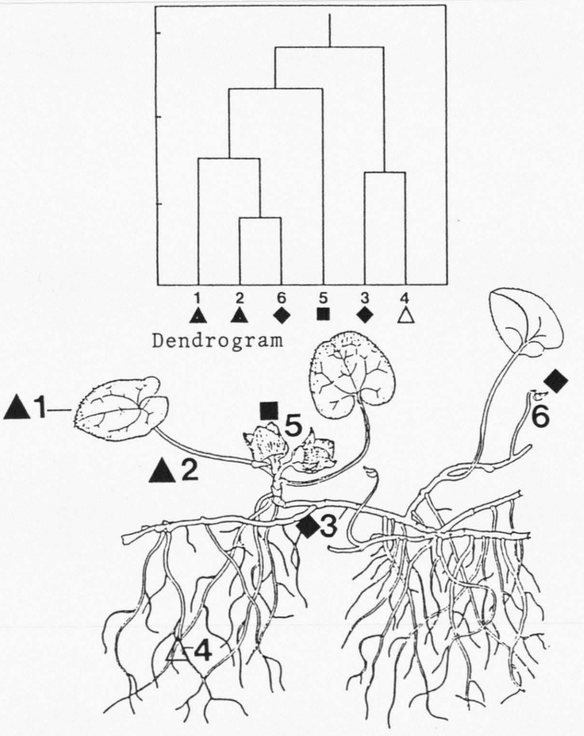

Fig. 5. The components of some organs of *Heterotropa takaoi* and dendrogram constructed from chemical parameters by the use of cluster analysis.

Temporal changes of the leaf terpenes and phenylpropanes of H. takaoi

Temporal changes of the volatile components of *H. takaoi* in Hiroshima were examined during six months (June, August, October, December). The leaves were analyzed from the current years growth throughout six months. Samples were collected at interval of two months from June to December. The sample thus included leaves of 0 to 6 months. The analytical results of temporal changes are shown in Table III. Although the components of leaves in June and December showed quantitative compositional differences, the components of the other two months were very similar to each other. The leaves in June contained large amounts of terpenes but smaller amounts of safrole rather than those of other three months.

Individual and age differences of H. takaoi

The results of the individual and age differences of the volatile components of the leaves of *H. takaoi* in Hiroshima are shown in Tables IV and V. The terpene and phenylpropane profiles of the individuals are very similar to each other.

Table III. Temporal changes of the volatile components of leaves of *Heterotropa takaoi* (from Hiroshima, population No. 1).

Component	Month June	August	October	December
α-pinene	4.6	1.0	1.4	1.1
camphene	2.1	0.5	0.5	0.4
β-pinene	15.6	4.9	6.9	7.4
p-cymene	3.2	1.6	1.1	0.7
myrcene	1.2	0.7	0.7	0.7
limonene	2.0	1.0	1.3	1.0
1,8-cineole	2.4	1.5	2.0	1.4
linalool	0.9	1.0	0.9	0.5
safrole	42.1	63.4	62.6	54.9
β-farnesene	8.6	5.7	6.5	6.5
α-bergamotene	4.0	3.6	3.9	4.5
caryophyllene	7.8	10.0	8.5	15.7
myristicin	2.5	2.9	1.5	1.0
n-pentadecane	–	0.6	0.5	0.7
elemicin	3.0	1.0	1.2	3.5
1-allyl-2,3,4,5-tetramethoxybenzene	–	0.1	0.5	–
terpene	52.4	31.5	33.7	39.9
phenylpropane	47.6	67.4	65.8	59.4
n-pentadecane	–	0.6	0.5	0.7

Table IV. Individual variation of the volatile components of leaves of *Heterotropa takaoi* (from Hiroshima, population No. 1).

Component	Individual 1	2	3	4	5	6	χ	σ
α-pinene	1.3	1.4	3.0	0.6	1.3	1.6	1.5	0.7
camphene	1.6	0.5	2.7	0.4	1.1	1.2	1.3	0.8
β-pinene	5.5	7.4	8.9	5.0	8.2	9.4	7.4	1.6
p-cymene	2.3	2.6	1.5	1.0	3.4	1.8	2.1	0.8
myrcene	1.6	1.4	1.7	0.9	0.6	1.3	1.3	0.4
limonene	1.0	2.0	1.6	1.0	1.4	1.3	1.4	0.8
1,8-cineole	1.3	2.5	2.0	1.2	2.6	2.0	1.9	0.8
linalool	0.9	1.6	0.8	0.7	0.9	1.0	1.0	0.3
safrole	69.5	60.5	59.2	63.6	58.1	62.8	62.3	3.7
β-farnesene	4.9	5.5	7.0	5.8	6.2	5.3	5.8	0.7
α-bergamotene	2.0	3.0	3.1	3.6	3.2	2.8	3.0	0.5
caryophyllene	3.5	8.1	5.4	11.2	6.9	6.1	6.9	2.4
myristicin	2.4	2.5	1.1	2.8	3.8	1.3	2.3	0.9
n-pentadecane	0.1	–	–	0.6	0.4	0.4	0.3	0.2
elemicin	1.4	1.0	2.1	0.9	1.3	1.2	1.2	0.5
1-allyl-2,3,4,5-tetra-methoxybenzene	0.7	–	–	0.7	0.6	0.5	0.3	0.3
terpene	25.9	36.0	37.6	31.4	35.8	33.8	33.9	
phenylpropane	74.0	64.0	62.4	68.0	63.8	65.8	66.1	
n-pentadecane	0.1	–	–	0.6	0.4	0.4	0.3	

Table V. Comparison of the volatile components of three different age classes of leaves of *Heterotropa takaoi* (from Hiroshima, population No. 1).

Component	Age class (year) (1–5)	(6–10)	(11–15)
α-pinene	1.4	1.8	1.8
camphene	0.5	0.8	0.9
β-pinene	7.5	8.2	9.0
p-cymene	0.8	1.4	2.6
myrcene	1.2	1.2	0.7
limonene	1.3	1.5	1.4
1,8-cineole	1.7	2.0	1.7
linalool	0.8	0.9	1.1
safrole	61.1	59.9	49.8
β-farnesene	7.7	6.8	9.3
α-bergamotene	4.2	3.9	4.4
caryophyllene	8.4	8.6	10.1
myristicin	1.4	1.8	4.1
n-pentadecane	0.3	0.2	2.9
elemicin	1.2	0.9	0.7
1-allyl-2,3,4,5-tetra-methoxybenzene	0.5	0.1	0.5
terpene	35.5	37.1	42.0
phenylpropane	64.2	62.7	55.1
n-pentadecane	0.3	0.2	2.9

The leaves of the young age class (1–5 years old) contained a small amount of terpenes but contained a large amount of phenylpropanes. On the other hand, the old age class (11–15 years old) contained a lot of terpenes but contained a small amount of safrole. The phenylpropane content was the greatest during the young age class and gradually declined to the low at old age class.

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