# Chemosystematics of Japanese *Heterotropa* (Aristolochiaceae)

Nanao Hayashi, Kazuyuki Maeshima, Tadayuki Murakami, and Hisashi Komae

Study of Environmental Science, Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima 730, Japan

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A survey of chemical composition of forty-four species of *Heterotropa* growing in Japan and Formosa showed that four chemical groups could be distinguished and that two routes of distribution to Japan from China may be suspected on the basis of phenylpropane components.

## Introduction

The genus *Asarum* of Aristolochiaceae with seventy species has a wide distribution in Asia, Europe, and North America. According to Engler's classification [1], all species are placed in one genus. In contrast, according to Maekawa's system [2], Asiatic species of the genus are divided into three genera, *Heterotropa* (ca. 50 species), *Asarum* (one species), and *Asiasarum* (three species). In this work, Maekawa's classification was used as genus *Heterotropa.* Although it is difficult to distinguish each species belonging to *Heterotropa* on morphological data, Maekawa reported about 50 species for this group in Japan.

From the viewpoint of karyomorphology, Ono [3] examined 22 species of *Heterotropa* ($2n = 24$) together with three species of *Hexastylis* from North America ($2n = 26$), one species of *Asarum* ($2n = 26$) from Canada, and one species of *Asiasarum* ($2n = 26$) from Japan. As for polyploidy, four species were found in *Heterotropa; H. megacalyx* ($2n = 48$), *H. rigescens* ($2n = 48$), *H. subrigescens* ($2n = 48$), *H. serpens* ($2n = 36$). In *H. regescens,* diploid ($2n = 24$) were observed together with the tetraploid ones ($2n = 48$), even in the same individual.

Phytochemical study of *Heterotropa* was first done by Nagasawa [4]. He analyzed the essential oils of eleven species by infrared spectroscopy and suggested the presence of safrole, elemicin, and limonene in the oils. Saiki [5] reported gas chromatographic analysis of phenolether and terpene components of 34 species of *Heterotropa* and found ten phenolethers and one monoterpene alcohol (borneol) in the oils. In the course of phytochemical study of *Heterotropa,* we have already investigated the components of 37 species [6] by means of gas chromatograph-mass spectrometry (GC-MS). In order to throw more light on the chemical variation of *Heterotropa,* seven species of the same genus in Japan were investigated and some chemosystematic aspects of the genus are discussed on the basis of the phytochemical data.

Reprint requests to N. Hayashi.

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## Experimental

### *Plant Materials*

The following forty-four species of *Heterotropa* were examined. Each species is given an accession code number in Table I and Figure 1. The localities at which the plants were collected are shown in Figure 1. (Code No. 1) *H. tamaensis* F. Maekawa, (2) *H. muramatsui* (Makino) F. Maekawa, (3) *H. savatieri* Franch, (4) *H. koyana var. nipponica* (F. Maekawa) Kitam., (5) *H. curvistigma* F. Maekawa, (6) *H. kurosawae* F. Maekawa, (7) *H. megacalyx* F. Maekawa, (8) *H. yoshikawae* F. Maekawa, (9) *H. nipponica var. brachypodion* F. Maekawa, (10) *H. nipponica var. rigescens* F. Maekawa, (11) *H. takaoi* F. Maekawa, (12) *H. costata* F. Maekawa, (13) *H. nankaiensis* F. Maekawa, (14) *H. sakawana* (Makino) F. Maekawa, (15) *H. stellatum* F. Maekawa, (16) *H. aspera* F. Maekawa, (17) *H. hexaloba* F. Maekawa, (18) *H. nomurana* F. Maekawa, (19) *H. asaroides* Morren et Decaisne, (20) *H. kuranarii* F. Maekawa, (21) *H. kiusiana* F. Maekawa, (22) *H. unzen* F. Maekawa, (23) *H. controversa* F. Maekawa, (24) *H. subglobosa* F. Maekawa, (25) *H. minamitaniana* Hatsusima, (26) *H. perfecta* F. Maekawa, (27) *H. satsumensis* F. Maekawa, (28) *H. trigina* (F. Maekawa) Araki, (29) *H. kumageana var. satakeana* F. Maekawa, (30) *H. kumageana* (Masamune)

F. Maekawa, (31) *H. yakushimensis* Masamune, (32) *H. tokarensis* Hatusima, (33) *H. gusuk* F. Maekawa, (34) *H. celsa* F. Maekawa, (35) *H. lutchuensis* (T. Ito) Honda, (36) *H. fudsinoi* (T. Ito) F. Maekawa, (37) *H. trinacriformis* F. Maekawa, (38) *H. hatusimae* F. Maekawa, (39) *H. similis* F. Maekawa, (40) *H. okinawensis* (Hatusima) F. Maekawa, (41) *H. dissita* F. Maekawa, (42) *H. gelasina* (F. Maek) F. Maekawa, (43) *H. yaeyamensis* (Hatusima) F. Maekawa, (44) *H. hayatana* F. Maekawa.

*Isolation and identification of the components of the essential oils*

The fresh whole plants (50–250 g) were collected during flowering season and chopped up, then, the cut materials were extracted with ethylacetate. The essental oils were obtained by evaporation of the solvent under nitrogen. The essential oils thus obtained were analyzed by GC-MS.

*Gas chromatography and GC-MS*

Quantitative GC analysis were carried out on a gas chromatograph 350 (Gasukuro Kogyo, Japan) which was combined with integrator and equipped with a 40 m × 0.28 mm glass capillary column coated with OV-101. The column temperature was programmed from 100 to 250 °C. GC-MS was measured with JMS-D-100 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. All mass spectra were obtained at 25 eV.

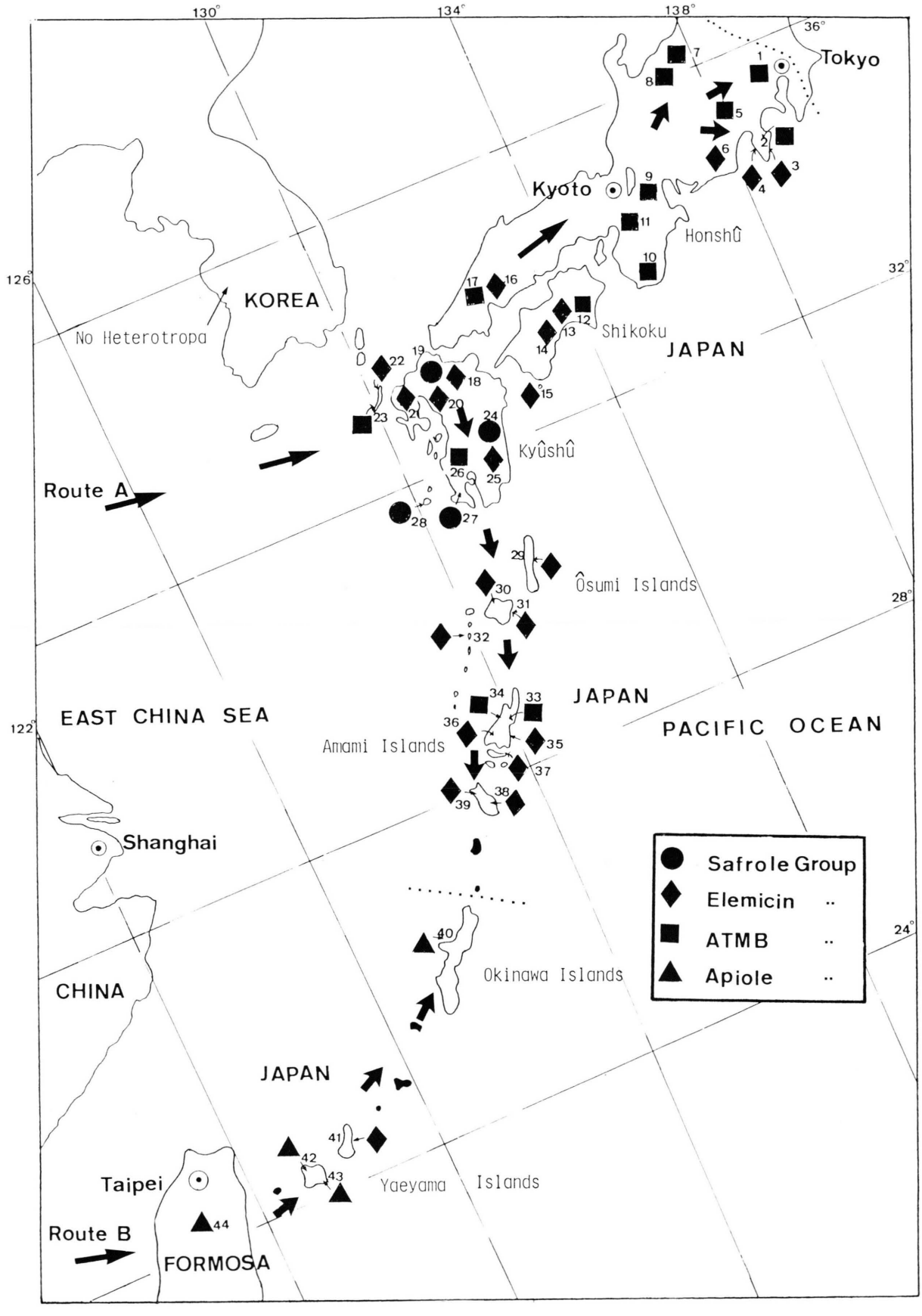
## Results and Discussion

The analytical results of seven species of *Heterotropa* examined in this study were as follows: *H. yoshikawae* F. Maekawa, leaves 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%), methyl eugenol (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%), 1-allyl-2,3,4,5-tetramethoxbenzene (ATMB, 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%), methyl eugenol (2.3%), *n*-pentadecane (4.0%), elemicin (21.4%), 1-allyl-2,3,4,5-tetramethoxybenzene and its isomers (24.7%). *H. magacalyx* F. Maekawa, leaves *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*-pentane (trace), *E*-β-farnesene (1.5%), α-curcumene (0.5%), α-cedrene (0.5%), elemicin (25.0%), 1-allyl-2,3,4,5-tetramethoxybenzene (22.0%). roots α-pinene (8.4%), camphene (6.1%), β-pinene (6.9%), myrcene (4.1%), camphor (2.8%), borneol (9.9%), bornyl acetate (1.5%), methyl eugenol (4.1%), α-bergamotene (1.6%), γ-elemene (2.9%), *n*-pentadecane (10.7%), elemicin (15.2%), 1-allyl-2,3,4,5-tetramethoxybenzene and its isomers (18.0%), asarone (5.9%). *H. nipponica var. rigescens* F. Maekawa, leaves α-pinene (0.5%), camphene (0.2%), β-pinene (1.0%), myrcene (trace), 1,8-cineole (0.6%), linalool (1.0%), borneol (trace), α-terpineol (trace), safrole (16.5%), methyl eugenol (4.3%), methyl isoeugenol (3.6%), α-humulene (14.6%), caryophyllene (5.0%), δ-selinene (25.0%), elemicin (8.6%), 1-allyl-2,3,4,5-tetramethoxybenzene (3.7%), asarone and its isomer (10.3%). roots α-pinene (2.2%), camphene (1.7%), β-pinene (4.8%), 1,8-cineole (1.0%), camphor (0.6%), borneol (0.8%), α-terpineol (0.7%), 3,4,5-trimethoxytoluene (1.7%), elemicin (50.5%), 1-allyl-2,3,4,5-tetramethoxybenzene (34.8%), asarone and its isomers (0.9%). *H. kurosawae* F. Maekawa, Leaves myrcene (trace), 1,8-cineole (trace), myristicin (98.2%). roots α-pinene (5.0%), camphene (3.0%), β-pinen (3.3%), 1,8-cineole (1.3%), borneol (4.4%), 3,4,5-trimethoxytoluene (18.5%), safrole (1.0%), *n*-pentadecane (2.0%), elemicin (61.5%). *H. nipponica var. brachypodion* F. Maekawa, leaves β-elemene (0.2%), caryophyllene (2.2%), α-bergamotene (9.4%), α-humulene (6.7%), methyl eugenol (0.2%), *n*-pentadecane (27.0%), elemicin (42.0%), asarone (7.5%). roots α-pinene (1.7%), camphene (1.4%), β-pinene (2.8%), camphor (0.3%), borneol (2.8%), bornyl acetate (0.3%), 3,4,5-trimethoxytoluene (1.0%), *n*-pentadecane (7.0%), elemicin (78.4%), asarone (0.3%), 1-allyl-2,3,4,5-tetramethoxybenzene (2.8%). *H. kura-*

Table I. Terpene and Phenolether Composition of *Heterotropa*.

| Code No. | *Heterotropa* | Eugenol | Safrole [a] | Elemicin [b] | ATMB [c] | Apiole | Terpene [d] | Orcinol [e] | Location |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *H. tamaensis* | 1.1 | 2.1 | 41.6 | 7.2 | 0 | 48.0 | 0 | |
| 2 | *H. muramatsui* | 0 | 8.8 | 21.8 | 29.7 | 0 | 39.7 | 0 | |
| 3 | *H. savatieri* | 0 | 2.2 | 57.6 | 0 | 0 | 36.6 | 3.6 | |
| 4 | *H. koyana var. nipponica* | 0 | 25.9 | 56.1 | 0 | 0 | 16.6 | 1.4 | |
| 5 | *H. curvistigma* | 0 | 3.5 | 23.9 | 3.2 | 0 | 55.0 | 14.4 | |
| 6 | *H. kurosawae* | 0 | 0.5 | 79.8 | 0 | 0 | 9.0 | 0 | Central Honshû |
| 7 | *H. megacalyx* | 0 | 2.0 | 23.1 | 20.0 | 0 | 47.1 | 0 | |
| 8 | *H. yoshikawae* | 0 | 2.2 | 35.7 | 13.6 | 0 | 38.8 | 0 | |
| 9 | *H. nipponica var. brachypodion* | 0 | 0.1 | 60.4 | 1.4 | 0 | 13.9 | 0.5 | |
| 10 | *H. nipponica var. rigescens* | 0 | 4.0 | 35.2 | 19.2 | 0 | 29.8 | 0.8 | |
| 11 | *H. takaoi* | 0 | 1.1 | 73.4 | 0 | 0 | 23.9 | 1.6 | |
| 12 | *H. costata* | 0 | 19.6 | 4.6 | 47.0 | 0 | 28.8 | 0 | |
| 13 | *H. nankaiensis* | 0 | 37.3 | 20.0 | 0 | 0 | 35.0 | 7.7 | |
| 14 | *H. sakawana* | 0 | 3.7 | 1.8 | 0 | 0 | 94.5 | 0 | Shikoku |
| 15 | *H. stelleta* | 0 | 13.1 | 4.9 | 0 | 0 | 82.0 | 0 | |
| 16 | *H. aspera* | 0 | 4.9 | 10.2 | 0 | 0 | 84.9 | 0 | West Honshû |
| 17 | *H. hexaloba* | 0 | 15.8 | 55.5 | 1.9 | 0 | 25.8 | 0 | |
| 18 | *H. nomurana* | 0 | 84.7 | 1.5 | 0 | 0 | 5.0 | 0 | |
| 19 | *H. asaroides* | 1.1 | 77.5 | 0 | 0 | 0 | 21.4 | 0 | |
| 20 | *H. kuranarii* | 0.4 | 63.8 | 16.5 | 0 | 0 | 18.2 | 0 | |
| 21 | *H. kiusiana* | 0 | 65.9 | 4.9 | 0 | 0 | 23.0 | 6.2 | |
| 22 | *H. unzen* | 0 | 17.6 | 38.2 | 0 | 0 | 44.2 | 0 | |
| 23 | *H. controversa* | 0 | 0 | 59.1 | 3.9 | 0 | 37.0 | 0 | |
| 24 | *H. subglobosa* | 0 | 83.9 | 0 | 0 | 0 | 16.1 | 0 | |
| 25 | *H. minamitaniana* | 0 | 0 | 2.9 | 0 | 0 | 97.1 | 0 | Kyûshû |
| 26 | *H. perfecta* | 0 | 0 | 10.9 | 5.2 | 0 | 68.4 | 15.5 | |
| 27 | *H. satsumensis* | 1.0 | 68.7 | 0 | 0 | 0 | 30.3 | 0 | |
| 28 | *H. trigina* | 0 | 0 | 77.6 | 0 | 0 | 22.4 | 0 | |
| 29 | *H. kumageana var. satakeana* | 0 | 0 | 97.0 | 0 | 0 | 3.0 | 0 | Ôsumi Islands |
| 30 | *H. kumageana* | 0 | 0 | 89.8 | 0 | 0 | 10.2 | 0 | |
| 31 | *H. yakusimensis* | 0 | 0 | 61.4 | 0 | 0 | 38.6 | 0 | |
| 32 | *H. tokarensis* | 0 | 1.0 | 91.4 | 0 | 0 | 7.5 | 0 | |
| 33 | *H. gusuk* | 0.5 | 6.3 | 20.9 | 2.0 | 0 | 70.2 | 0 | |
| 34 | *H. celsa* | 0 | 0.6 | 25.5 | 1.8 | 0 | 71.4 | 0.7 | |
| 35 | *H. lutchuensis* | 0 | 4.1 | 55.4 | 0 | 0 | 40.5 | 0 | |
| 36 | *H. fudsinoi* | 0.5 | 61.0 | 30.1 | 0 | 0 | 8.4 | 0 | Amami Islands |
| 37 | *H. trinacriformis* | 0 | 0 | 66.6 | 0 | 0 | 33.3 | 0.2 | |
| 38 | *H. hatushima* | 0 | 1.8 | 47.5 | 0 | 0 | 49.0 | 1.7 | |
| 39 | *H. similis* | 0 | 0.7 | 33.9 | 0 | 0 | 65.0 | 0.4 | |
| 40 | *H. okinawensis* | 0 | 0 | 14.8 | 0 | 43.4 | 41.3 | 0 | Okinawa |
| 41 | *H. dissita* | 0 | 39.1 | 40.2 | 0 | 0 | 17.8 | 0 | |
| 42 | *H. gelasina* | 0 | 52.3 | 18.8 | 0 | 6.7 | 21.3 | 0 | Yaeyama Islands |
| 43 | *H. yaeyamensis* | 0 | 0.9 | 22.5 | 0 | 47.3 | 31.3 | 0 | |
| 44 | *H. hayatana* | 0 | 0 | 24.6 | 0 | 8.7 | 61.9 | 0 | Formosa |

[a] safrole and eugenol methylether.
[b] elemicin, myristicin and asarone.
[c] 1-allyl-2,3,4,5-tetramethoxybenzene.
[d] mono- and sesquiterpene.
[e] 3,5-dimethoxytoluene and 3,4,5-trimethoxytoluene.


130°
134°
138°
36°
32°
28°
24°
126°
122°
Tokyo
Kyoto
Honshû
KOREA
No Heterotropa
Shikoku
JAPAN
Kyûshû
Route A
Ôsumi Islands
EAST CHINA SEA
JAPAN
PACIFIC OCEAN
Amami Islands
Shanghai
CHINA
Okinawa Islands
JAPAN
Taipei
Yaeyama Islands
Route B
FORMOSA
Safrole Group
Elemicin ..
ATMB ..
Apiole ..

*narii* F. Maekawa, leaves myrcene (3.0%), safrole (35.2%), methyl eugenol (48.3%), methyl isoeugenol (3.8%), *E*-β-farnesene (9.5%). roots α-pinene (6.6%), camphene (3.5%), β-pinene (10.7%), myrcene (1.6%), borneol (2.1%), eugenol (0.8%), safrole (5.3%), methyl eugenol (34.9%), elemicin (32.1%), asarone (0.9%). *H. nomurana* F. Maekawa, leaves α-pinene (1.2%), β-pinene (2.0%), safrole (45.1%), methyl eugenol (24.7%), methyl isoeugenol (20.3%), elemicin (3.1%). roots α-pinene (2.1%), camphene 1.2%, β-pinene (3.5%), 3,5-dimethoxytoluene (0.7%), safrole (7.8%), methyl eugenol (71.6%), elemicin (12.9%).

The distribution of phenolether and terpene components in the oils of seven species in addition to 37 species previously reported is shown in Table I. The main components of all *Heterotropa* species were terpene, elemicin, safrole, ATMB, and apiole. In order to discuss the chemotaxonomy of *Heterotropa*, four characteristic phenylpropanes (safrole, elemicin, ATMB, apiole) were used as chemosystematic markers. Fig. 2 showed the oxidation level of the phenylpropane components. The oxidation level increases from safrole to ATMB level. Gottlief [7] reported that micromolecules evolution involves changes in oxidation level and the direction of evolution may be increased in oxidation level. Based on the oxidation level of the phenylpropane components, we divided them into four chemical groups (safrole, elemicin, ATMB, and apiole groups). Although pathways A and B in Fig. 2 sometime occurs in the same plants except apiole and ATMB. We could not isolate both apiole and ATMB from the same plants. Pathway A occurs in the leaves more than roots, while pathway B occurs in the roots more than leaves as shown in the present work. Geographical distribution of phenylpropanes in *Heterotropa* are shown in Fig. 1. A large quantity of safrole occurs in the species distributed in Kyushu Island. Elemicin is distributed in the species of Ôsumi and Shikoku Islands adjacent to Kyushu. ATMB is distributed in the species of Northeast of Honshu, where is the northernmost tip of *Heterotropa* distribution. In contrast, apiole was distributed in southern Islands of Japan. It is well known that *Heterotropa* species are originated from Yunnan or Szechuan Province in China. The above results suggest that Japanese *Heterotropa* seem to have radiated from China to Japan through two routes (routes A and B) as shown in Fig. 1.

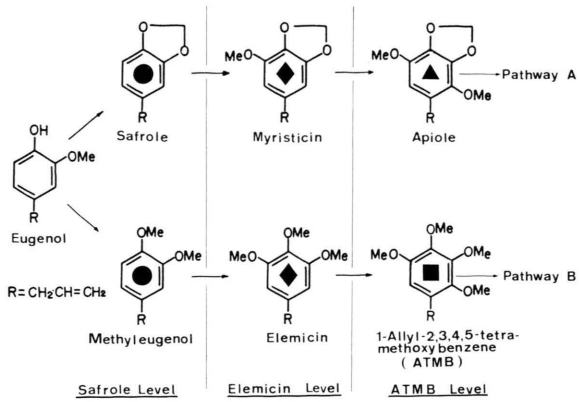



Fig. 2. Oxidation level of the phenol ethers in *Heterotropa*.

*H. megacalyx* ($2n = 48$) and *H. regescens* ($2n = 48$ and $2n = 24$) which may be regarded as highly advanced species contained large amounts of the most oxygenated phenylpropane, ATMB in the oils. *H. yoshikawae* ($2n = 24$) which occurs in adjacent areas of *H. megacalyx*, also contained similar phenylpropanes. The variation of chromosome number may be of no effect to the chemical components. These results supported by the fact that American *Hexastylis* ($2n = 26$) closely related to the genus *Heterotropa* contained similar chemical components (methyl eugenol, safrole, terpene, and elemicin) [8].

Fig. 1. Geographical distribution of *Heterotropa* species with phenylpropanes.

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