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Kyoto University
Some Chemical Observation on a Natural Graft-Wood of Japanese Cypress (Chamaecyparis obtusa Endl.) and Sawara Cypress (Chamaecyparis pisifera Sieb. et Zucc.)*

Akira Sato**, Teruko Kitamura** and Takayoshi Higuchi**

Abstract—Chemical analyses were carried out on a graft-wood of hinoki (Chamaecyparis obtusa) and sawara cypress (C. pisifera), and the results were compared with the data from normal woods of hinoki and sawara. The amount of boiling water soluble matter were much higher in the graft-wood than in normal woods (1.5-3.6 times), and also the amount of alcohol-benzene soluble matter in the graft-woods were two times higher than those of the normal woods. Lignin content varied little among the samples. α-Terpinol which is the most characteristic component to identify hinoki and sawara woods, borneol, DL-limonene and bornyl acetate were identified, respectively. Some group peaks which are supposed to be cadinenes and cadinols were also found on g.l.c. of steam volatile fraction. Sawaranin [2,3-desoxy-2-(p-hydroxy phenyl hexonic lactol)] was first isolated from methanol extracts of normal woods of hinoki and identified with the results of u.v., i.r. and n.m.r.

Introduction

Hinoki (Chamaecyparis obtusa Endl.) and sawara (Chamaecyparis pisifera Sieb. et Zucc.) cypresses are closely related in species and a natural graft of both woods has been discovered at the National Forest, Tsukechi District, Gifu prefecture. The authors have recently obtained such a graft-wood of hinoki and sawara shown in Fig. 1 from the National Forest.

In the wood of hinoki (Japanese cypress), the following chemical compounds have been found; α-pinene, thujopsene, cadinene, cadinol, hinokiol, hinokione, hinokinine (cubein) and hinokiresino. On the wood of sawara cypress the occurrence of the following compounds as well as some of the above terpenoids has been reported; sawaranin [2,3-desoxy-2-(p-hydroxy phenyl hexonic lactol)], arabinose, potassium hydrogen oxalate and savinin (hibalactone).

It is interesting at the standpoint of chemotaxonomy and comparative biochemistry to know the distribution of chemical constituents in such a graft-wood in relation to the possible variation of the constituents of each species.

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** Division of Lignin Chemistry.
In the present experiment, ordinary chemical analysis of the hinoki and sawara parts of the graft-wood and normal woods of both cypresses have been carried out. Although hinokinin, savinin, hinokiresinol and sawaranin which were considered as suitable indices for the above described purpose, none of them were identified through this experiment except sawaranin. However, sawaranin was found in normal wood of hinoki, unexpectedly.

**Results and Discussion**

The mean values of dual or triple analyses of moisture, boiling water soluble matter, alcohol-benzene soluble matter and lignin are given in Table 1. Chemical compositions of normal woods were generally reasonable comparing with those

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<th>Hinoki</th>
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<td>Moisture (%)</td>
<td>18.3</td>
<td>14.2</td>
</tr>
<tr>
<td>Boiling water soluble (%)</td>
<td>1.7</td>
<td>6.2</td>
</tr>
<tr>
<td>Alcohol-benzene soluble (%)</td>
<td>8.3</td>
<td>17.3</td>
</tr>
<tr>
<td>Lignin (%)</td>
<td>31.0</td>
<td>29.7</td>
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reported in old literatures\textsuperscript{5,6}, except the quantities of alcohol-benzene soluble matter were about two times higher. On the other hand, contents of boiling water soluble matter and alcohol benzene soluble matter differed considerably between the normal and the graft-wood. For instance, the contents of boiling water soluble matter in the graft-wood showed 3.6 and 1.4 times higher than those of sawara and hinoki normal woods, respectively, whereas the difference in the graft-wood between hinoki and sawara parts was minute. The contents of the alcohol-benzene soluble matter from each part of the graft-wood were almost two times higher than those of the normal woods, and that from the part of sawara wood was slightly higher than that from hinoki. Such higher value is seemed to be ascribed to an abnormal conjunctive tissue. However, lignin contents were quite similar among the samples.

Gas-liquid chromatograms of the steam volatile fraction from each normal and graft-wood are shown in Fig. 2 and 3, respectively. Sensitivity range of the instrument was switched over to half at the point of mark S on the chromatograms. As shown in Fig. 2, two groups of peaks which appeared over 200°C were detected. It is suggested that the former at lower temperature contains cadinenes and the latter of higher temperature does cadinols. Other peaks at lower temperature from 80°C to 200°C, were established to be monoterpenes and the peaks of $\alpha$-terpineol and borneol were identified in both woods. Minor peaks of DL-limonene and bornyl acetate were also identified in sawara. Main peaks due to $\alpha$-terpinol and another unidentified peak which appeared around 190°C were characteristic in sawara.

![Fig. 2. Gas-liquid Chromatograms of the Volatile Oil from Normal Wood of Hinoki and Sawara.](image-url)

(1) borneol (2) DL-limonene (3) $\alpha$-terpineol (4) bornyl acetate (5) sesquiterpenes (cadinenes) (6) sesquiterpenealcohols (cadinols)
Generally, the group of cadinenes was more contained than cadinols in both woods.

In the case of the graft-wood, chromatograms of both hinoki and sawara parts separated kept characteristics of each original wood as shown in Fig. 3, although the pattern of chromatograms was similar to each other.

![Fig. 3. Gas-liquid Chromatograms of the Volatile Oil from a Graft-Wood of Hinoki and Sawara.](image)

Acetone extracts were analyzed by t.l.c. Each sample solution was prepared from either n-hexane insoluble or soluble fraction after removing steam volatile matter, and the fractions from normal woods thus obtained were fractionated by 1% sodium hydroxide solution. The results are summarized in Fig. 4. The chromatogram developed with chloroform-acetic acid and colored with diazotized

![Fig. 4. Thin-layer Chromatograms of the Ether Soluble Matters.](image)
benzidine is shown in Fig. 4. By this solvent, the spot of sawaranin did not move from the original point. The spots of hinokinin, savinin and a few kinds of flavonoids were supposed to be present on the chromatogram, although each spot was not yet fully identified. Only main difference between the normal woods of sawara and hinoki was of the spot marked C and D in Fig. 4. For instance, spot C which gives Rf value of 0.30 appeared only in hinoki, but spot D of Rf value 0.40 did only in sawara wood. Differences between the graft-wood and the normal woods were generally quite small. Spot H showed a little difference in hinoki, spot D in the normal sawara disappeared and spot F newly appeared in the sawara part of the graft-wood. Such similarity is not surprised as both species belong to the same genus. Thus, the special pattern of the extractives was not found in the graft-wood, and the hinoki and sawara parts of the graft-wood generally gave chemical components of the respective normal woods.

Pale yellow crystals were isolated as flakes from the methanol extract of the
normal hinoki wood. This compound was eluted with solvents containing acetone from silicic acid column, recrystalized with boiling water and melted at 212-214°C. Elementary analysis was coincident with formula $C_{12}H_{14}O_6$ and spectrum of u.v. absorption showed Max. at 277 nm in 95% EtOH. The absorption spectra of i.r. and n.m.r. measured with the solution of deuterio DMSO were also obtained and the results were coincident with those of authentic sawaranin. The i.r. and n.m.r. charts are shown in Fig. 5 and 6, respectively. Thus it was established that sawaranin was not only distributed in the wood of sawara, but also in the wood of hinoki, and the fact was very interesting from the view point of chemotaxonomy of genus, Chamaecyparis.

Experimental

Samples of a natural graft-wood between hinoki and sawara, and respective normal woods as standard were collected from Tsukechi District, Gifu Prefecture. Wood meal named grafting hinoki or sawara were prepared from the each part of the wood using Willey’s mill, respectively.

Wood meal from the normal wood of both species which mainly composed of heartwood was also prepared with Willey’s mill and named as normal hinoki and sawara.

Each sample wood was used for chemical analyses to determine, moisture, boiling water extract, alcohol-benzene extract and lignin by the methods of JIS standard; namely, JIS P-8002, P-8005, P-8010 and P-8008, in order.

Steam Distillation Two hundreds grams of wood meal were subjected to steam distillation for two hours. The distillate caught in ice cold acetone was transferred to ether layer as usual, and the concentrated ether solutions were applied for g.l.c.

Gas Liquid Chromatography (g.l.c.) The instrument, JBOl 750 fixed with 2 meters of 10% Apiezon N on chromosorb W (3 mm φ stainless steel) was operated with temperature range 80°C to 240°C, in the atmosphere of helium, and compounds were detected with TCD. Monoterpenes were identified comparing with retention times of the authentic ones.

Thin-Layer Chromatography (t.l.c.) Glass plates coated with Silica gel G (Merck) were used for ether soluble fractions and developed with chroloform-acetic acid (9:1 v/v) as shown in Fig. 4. Diazotized benzidine was used as a coloring reagent. The fraction named St. NaOH was once extracted with 1% sodium hydroxide.

Samples for the t.l.c. were prepared from acetone extract by using a Soxhlet’s apparatus.

The i.r. charts were obtained using KBr pellets with JASCO IR-S. U.v. chart
was recorded by using Hitachi 120 double beam spectrophotometer in 95 % ethanolic solution. Charts of n.m.r. were taken in the 10 % solution of D<sub>6</sub>-DMSO at 22°C using JEOL, Minimer (MH-60, 60 MHz).

As extractives for isolating sawaranin the ether soluble parts of methanol soluble matters of normal hinoki were chosen.

For instance, five grams of methanol soluble and ether soluble fractions from normal hinoki were placed on the top of silicic acid column (Merck, 0.05-0.2 mm; 10 mmφ, 30 cm long) packed with chloroform, and following solvent systems were applied for elution, in order; ether-chloroform (1:1 v/v, 300 ml), chloroform (150 ml), chloroform-acetic acid (9:1 v/v, 500 ml) and acetone-chloroform-acetic acid (50:45:5 v/v, 500 ml). Crude crystals (217 mg) were collected from a red-brownish solution of the eluate with the above last solvent system. The solvent system of ethylacetate-acetone (1:1 v/v), instead of the above acetone-chloroform-acetic acid system, was also available to obtain the eluate in which sawaranin was contained. The above described crystals were established to be sawaranin in comparison with the data of λ<sub>max</sub>. in u.v. (277 nm in 95 % ethanol) and the identity of i.r. chart with authentic one as shown in Fig. 5. Recrystalized sawaranin was insoluble in ether and following results were obtained from the elementary analysis.

Found:  C., 56.48 %; H., 5.49 %
Calcd. for C<sub>12</sub>H<sub>14</sub>O<sub>6</sub>:  C., 56.68 %; H., 5.56 %

Acknowledgement

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Literature