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136. Yutaka Fujise, Isao Maruta, Shô Itô, and Tetsuo Nozoe: Chemical Constituents of Essential Oil of *Chamaecyparis Obtusa* (Sieb. et Zucc.)[*1]

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Chemical constituents of the neutral fraction of the essential oil of Japanese hinoki, *Chamaecyparis obtusa* (Sieb. et Zucc.), have already been investigated. The presence of *d*-[1,2] and *l*-[3] cadinenes, a cadinol,[2] an unidentified sesquiterpene hydrocarbon, an alcohol, and a diterpene hydrocarbon has been reported.[1,4] A study of the unidentified compounds was undertaken as part of our survey on the constituents of the plant of the Natural Order Cupressales in Japan.

Fractionation of the higher boiling portion of commercial hinoki leaf oil led to the isolation and identification of the following three compounds: *d*-Longifolene,[5] (60% of the oil) (hydrochloride, m.p. 59.5°,[*3] longidione, m.p. 94~98°[5]), *d*-γ-cadinene[6] (25%) (dihydrochloride,[*4] m.p. 114~116°), and thunbergene (cembrene) (0.8%) which was obtained as a viscous liquid after purification by preparative gas chromatography. Although this liquid could not be induced to crystallize, the infrared and nuclear magnetic resonance spectra were identical with those reported for thunbergene.[7]

The presence of these rather unusual hydrocarbons, *d*-longifolene and thunbergene, both of which have been found in pinus species but not in the cupressaceae, prompted us further to examine the whole essential oil from the leaves of a properly identified hinoki tree. Fractionation of the essential oil from hinoki leaves[*5] gave the following monoterpenes which were identified by gas chromatography (see the Fig. 1): α-pinene 0.21%,

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[*1] Presented at the 7th Symposium on Terpene, Perfumery, and Essential Oil Chemistry, Kochi, October 13th, 1963.

[*2] Katahira-cho, Sendai (藤瀬 裕, 丸田 勳, 伊東 椒, 野副鉄男).

[*3] An authentic specimen was provided by Professor I. Ogura of Kinki University, to whom our thanks are due.

[*4] The identity was established by Dr. T. Sakai, Institute of Food Chemistry, Osaka. The authors are indebted to him for making this comparison.

[*5] The specimen was collected in Sendai and identified by Mr. S. Obata, Tohoku University, to whom authors thanks are due.

1) E. Guenther: "The Essential Oil," Vol., **6**, p. 341, Van Nostrand Co., New York.
2) N. Hirao: Nippon Kagaku Zasshi, **58**, 222 (1937).
3) R. Kondo: Yakugaku Zasshi, **334**, 1297 (1909).
4) S. Uchida: Kôgyô Kagaku Zasshi **31**, 650 (1928).
5) G. Dupont, R. Dulou, P. Naffa, G. Ourisson: Bull. soc. chim. France, **1954**, 1075; P. Naffa, G. Ourisson: *Ibid.*, **1954**, 1115. Authentic samples were provided by Professor Ourisson, University of Strassbourg, to whom the authors are grateful.
6) V. Sýkora, V. Herout: Collection Czechoslov. Chem. Communs., **24**, 1732 (1959).
7) W. Dauben, W.E. Thiessen, R.R. Resnick: J. Am. Chem. Soc., **84**, 2015 (1962). H. Kobayashi, S. Akiyoshi: Bull. Chem. Soc. Japan, **36**, 823 (1963). The authors are indebted to Dr. Kobayashi Kyushu University, for making the IR comparison.

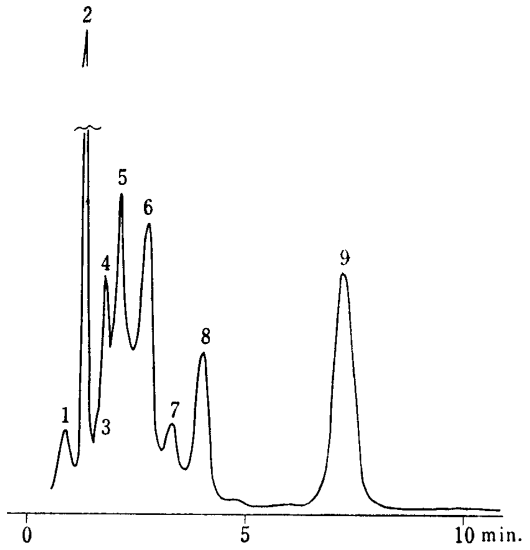

Fig. 1. Gas Chromatogram of Monoterpene Fraction of Hinoki Leaf Oil at 160°

1 : α-pinene 2 : α-thujone
3 : β-pinene 4 : limonene
5 : γ-terpinene 6~8 : unidentified
9 : bornyl acetate

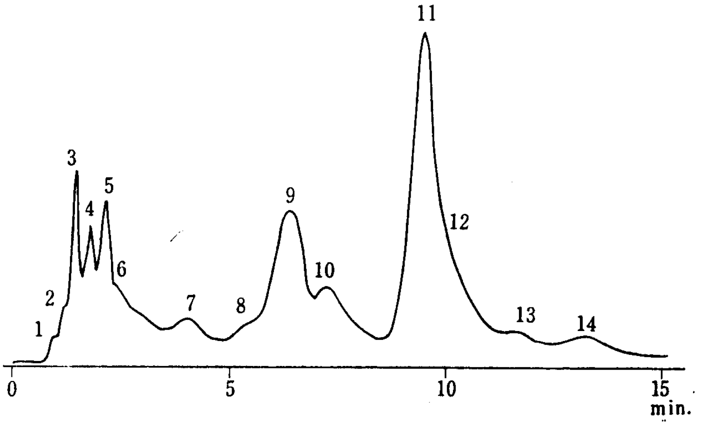

Fig. 2. Gas Chromatogram of Monoterpene Fraction of Hinoki Wood Oil at 160°

1 : camphene 2 : β-pinene 3 : α-terpinene
4 : γ-terpinene 5,6 : unidentified 7 : linalool monoxide
8 : linalool 9 : camphor 10 : bornyl acetate
11 : α-terpineol 12 : borneol 13 : geranyl acetate
14 : cadinene

α-thujone 2.08%, β-pinene a trace, limonene 0.53%, γ-terpinene 1.04%, bornyl acetate 8.10%, β-terpineol 0.96%, and borneol 0.85%. The distillation residue on alumina chromatography afforded elemol, m.p. 50°, identified by gas chromatography, infrared and nuclear magnetic resonance spectra, and by a mixed melting point measurement with an authentic specimen.[8] However, *d*-longifolene and γ-cadinene, the major components of the commercial oil, were not detected.

In the expectation that two compounds detected in the commercial oil might have originated from the wood, the essential oil obtained from hinoki saw-dust collected in the Nagano prefecture was next examined. The gas chromatogram (cf. Fig. 2) indicated the presence of the following terpenes : camphene 0.01%, β-pinene 0.05%, α-terpinene 0.15%, γ-terpinene 0.22%, linalool monoxide 0.17%, linalool a trace, camphor 0.43%, bornyl acetate 0.28%, α-terpineol and borneol 0.95%, geranyl acetate a trace, and γ-cadinene 42.6%. From the distillation residue, α-cadinol[9] and another cadinol of m.p. 77~77.5°, previously reported by Šorm,[9] were isolated.

After completion of our manuscript, a paper by Hayashi, *et al.*[10] appeared showing the presence of *d*-longifolene, *d*-γ-cadinene, calamenene, and *l*-α-cadinol in the essential oil of the leaves and twigs of the same plant collected in Kochi prefecture. By direct comparison,[*6] we were able to identify a trace of calamenene in our wood oil and in the commercial oil but not in the leaf oil.

The similarity between the constituents of the commercial leaf oil and those of Hayashi's sample, and the fact that then both differ from the constituents of our leaf oil, may be attributed to effect of climatic difference on the hinoki tree. If this is the case,

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[*6] Authors are greatly indebted to Professor Hayashi, Hiroshima University, for the generous donation of the calamenene fraction.

8) V. Sýkora, V. Herout, F. Šorm : Chem. Listy, 48, 1656 (1954). Thanks are due to Professor Herout for his generous gift of authentic elemol.

9) O. Motl, V. Sýkora, V. Herout, F. Šorm : Tetrahedron, 4, 246 (1958).

10) S. Hayashi, K. Yano, T. Matsuura : Bull. Chem. Soc. Japan, 37, 474 (1964).

it is probable that the commercial oil was obtained from trees grown in southern Japan where the hinoki tree is more abundant. A more detailed study is required to determine the effect of climatic differences on the constituents of hinoki.

Experimental

Melting points are uncorrected. $[\alpha]_D$s and UV spectra are refered to isooctane solution unless otherwise stated. IR spectra are for liquid film unless otherwise stated. NMR spectra were measured on a Varian A-60 spectrometer at 60 Mc. for CCl_4 solutions. Chemical shifts are expressed in p.p.m. from internal tetramethylsilane together with the appearance of signals (s : singlet, d : doublet, t : triplet, q : quartet) and number of hydrogen correspond to the signal. When used for identification, the gas chromatograms (VPC) were determined at different temperatures (100°, 120°, and 160°) with Hitachi KGL-2 Gas Chromatograph using 2 m. column filled with 25% of PEG 6000 on fire brick. The retention time (RT) in VPC is stated with the temperature used. The velocity of carrier gas, He, was always 60 ml./min.

Commercial Hinoki Leaf Oil——Material was kindly supplied by Takasago Perfumery Industry Co., Ltd. after removal of the monoterpene fraction by distillation. Further fractional distillation using a packed column afforded two main fractions.

d-Longifolene : 60% of the material, b.p_4 86.5°, n_D^{23} 1.4908, d_D^{20} 0.9325, $[\alpha]_D^{22}$ +33.2° (c=3.42, $CHCl_3$), RT 8.07 min. at 160°. IR ν_{max} cm^{-1} : 3082, 1652, 1098, 877. NMR δ : 0.90 (s, 3H), 0.95 (s, 3H), 1.00 (s, 3H), 4.73 (s, 1H), and 4.48 (s, 1H). All of these physical constants agree with those of authentic longifolene. Hydrochloride : m.p. 59.5°. The mixed melting point with longifolene hydrochloride was 59.5°. $[\alpha]_D^{8.5}$ $+84.4^c$ (c=1.05).

Longidione : Ten grams of the oil was ozonized in 100 ml. of $CHCl_3$ at −40°. After reductive decomposition of the ozonide and alumina chromatography, yellow prisms, m.p. 94~95°, were obtained. The IR spectrum was identical with that of longidione. No melting point depression was observed upon admixture of the sample with an authentic specimen.

d-γ-Cadinene : 25% of the oil, b.p_5 108~108.5°, n_D^{23} 1.4902, d_4^{20} 0.9260, $[\alpha]_D^{22}$ +28.3° (c=2.67, $CHCl_3$), RT 13.36 min. at 162°. UV : λ_{max} 246 mμ (ε 2025). IR ν_{max} cm^{-1} : 1670, 1650, 890, 837. IR spectrum is superimposable with that recorded for γ-cadinene. Dihydrochloride : m.p. 114~116°, $[\alpha]_D^{8}$ −42.27° (c=1.00). No depression was observed in melting point of the mixture of the sample and (−) cadinene dihydrochloride, whereas a marked depression was observed in the case of a mixture with (+) cadinene dihydrochloride. The IR spectrum is superimposable with authentic (−) cadinene dihydrochloride.

Cadalene picrate : One gram of the fraction was heated with the same amount of 5% Pd-C at 285° for 5 hr. under N_2 atmosphere. Extraction of the reaction mixture with benzene and CCl_4, followed by evaporation of the solvents, afforded 408 mg. of brown oil. Addition of picric acid in Et_2O solution and recrystallization of the precipitate from EtOH gave orange needles, m.p. 114~119.5°. The IR spectrum is superimposable with that recorded for an authentic specimen.

Thunbergene : 0.8% of oil. The residue from the above-mentioned distillation was further separated by preparative VPC using 2 m. column filled with fire brick coated with 25% of silicone oil DC-550 at 190°. The fraction with RT 40~50 min. was an oil; n_D^{11} 1.4908, d_4^{20} 0.9227, $[\alpha]_D^{7.2}$ +51.6° (c=1.00). UV : λ_{max} 246.5 mμ (ε 11018). IR ν_{max} cm^{-1} : 1670, 1160, 966, 840, 811. NMR δ : 0.91 (d, J=6 c.p.s., 6H), 1.50 (s, 3H), 1.59 (s, 3H), 1.77 (s, 3H), 4.3~6.2 (broad, 3H). These physical constants agree with those reported for thunbergene.

Leaf Oil——Hinoki leaves (3.0 kg.) collected in Tohoku University campus was cut into pieces and extracted at room temperature with MeOH for 19 days. After removal of the solvent, $CHCl_3$ was added, the solution washed successively with H_2O, 5% NaOH, and H_2O, and then dried. After evaporation of the solvent, the residue was steam distilled to yield 11.3 g. (ca. 0.4%) of neutral essential oil. Distillation of the oil through a short column gave the following three fractions : 1) colorless oil, 4.00 g., b.p_3 85°; 2) colorless crystals, 2.9 g., b.p_1 85~97°; 3) tarry residue, 4.63 g.

The first fraction was examined by VPC. The components were identified from their RT at different temperatures and by the increase in peak hights when an authentic sample was added. The results are shown in the first section of this paper.

Elemol : The crystalline fraction, after alumina chromatography and sublimation *in vacuo*, had m.p. 49.8° (no depression of melting point on admixture with authentic elemol), b.p_1 90°, $[\alpha]_D^{17.2}$ +2.8° (c=1.05, Me_2CO). IR ν_{max}^{KBr} cm^{-1} : 3374, 1635, 908, 892, 885. NMR δ : 0.90 (s, 3H), 1.14 (s, 6H), 1.68 (s, 3H), 4.71 (complex triplet, 4H), 5.72 (q, 1H).

Hinoki Wood Oil——Saw-dust (8.0 kg.) collected in Kiso district of Nagano prefecture was extracted repeatedly with MeOH at room temperature for 15 days. After similar treatment to that described above, the extract afforded 120 g. (1.5%) of neutral oil after evaporation of $CHCl_3$. The oil was separated by distillation into monoterpenic (1.25 g., b.p_{15} ~180° (bath)) and sesquiterpenic (56.0 g., b.p_2 111~160°)

fractions. The components of both fractions were identified by VPC as described in the previous section. The results are given in the earlier section.

A 2.6 g. portion of the distillation residue (62.0 g.) was redistilled at 0.5 mm. Hg through a short column until a bath temperature of 180° was reached. The distillate, after acetylation with Ac_2O and pyridine, and chromatography on alumina, afforded the following crystalline compounds. Cadinol, m.p. 77~77.5°, $[\alpha]_D^{21}$ −40.2° (c=1.6, CCl_4). IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1}: 3259, 1140, 908, 932, 887, 832. α-Cadinol, m.p. 40~60°, $[\alpha]_D^{21}$ −8.2° (c=0.75, CCl_4). IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1}: 3361, 1124, 1042, 1010, 950, 932, 895, 812. Both compounds were identified by comparison of their IR spectra with those of authentic specimens.

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Summary

The constituents of the essential oil from the leaf and wood of *Chamaecyparis obtusa* (Sieb. et Zucc.) have been investigated; a sample of the commercial leaf oil was also examined. Elemol and eight monoterpenes were found in the leaf oil. This is the first recorded isolation of elemol from this species. The wood oil was found to contain γ-cadinene, calamenene, two cadinols, and seven monoterpenes. *d*-Longifolene, *d*-γ-cadinene, calamenene, and thunbergene were identified in the commercial leaf oil.

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137. Keiji Sekiguchi, Toshihisa Yotsuyanagi,[*1] and Soichi Mikami[*2]: Studies on the Method of Thermal Analysis of Organic Medicinals. V.[*3,*4] Semi-micro Apparatus for the Differential Thermal Analysis Permitting Direct Observation.

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Although differential thermal analysis (DTA) has been developed since the end of the last century, applications were mainly limited to inorganic materials, such as ceramics, minerals and alloys, and rather few investigations concerning the thermal behaviors of organic materials were performed except those of natural and synthetic polymers. The reason why the method has been rarely applied to organic substances of low and medium molecular weight will be ascribed to the followings: 1. Because the apparatus is mostly designed for the metallurgical studies operating at higher temperatures, it does not fit well for use at lower range of temperature. 2. The sample size is usually about several hundred miligrams or more which will often be an extravagant requirement for organic chemists.

Since DTA is not applied so frequently as certain other kinds of measurement, such as spectrophotometry or pH determination significance will at present be attached to the mere collection of experimental data concerning thermal behaviors of organic materials.

[*1] Kita-12-Jo, Nishi-5-chome, Sapporo, Hokkaido (関口慶二, 四柳智久).

[*2] Hongo, Tokyo (三上総一).

[*3] Part Ⅳ. K. Sekiguchi, K. Ito, Y. Nakamori: This Bulletin, **11**, 1123 (1963).

[*4] Partly presented at the Hokkaido Branch Meeting of Pharmaceutical Society of Japan, August, 19th, 1962.