Holzforschung, Vol. 60, pp. 528–532, 2006 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/HF.2006.087

Analysis of fragrance compositions of precious coniferous woods grown in Taiwan

Sheng-Yang Wang*, Yen-Shung Wang, Yen-Hsueh Tseng, Chien-Tsong Lin and Chiung-Pin Liu

Department of Forestry, National Chung-Hsing University, Taichung, Taiwan

*Corresponding author.
Department of Forestry, National Chung-Hsing University, No. 250, Kuo-Kuang Road, Taichung 402, Taiwan
Phone: +886-4-22840345 ext 138
Fax: +886-4-22873628
E-mail: taiwanfir@dragon.nchu.edu.tw

Abstract

Odor is one of the most distinctive characteristics of wood. Woods in the family Taxodiaceae and Cupressaceae often emit fragrant odors. In this study, the fragrance compounds of six coniferous woods grown in Taiwan, namely *Chamaecyparis formosensis*, *Chamaecyparis obtusa* var. *formosana*, *Calocedrus macrolepis* var. *formosana*, *Taiwania cryptomerioides*, *Cunninghamia lanceolata*, and *Cryptomeria japonica*, were studied by solid-phase microextraction and GC/MS. A non-biased overall profile of the fragrance compositions of the woods was obtained. The major aroma compounds were: β-elemene (15.8%), γ-cadinene (12.1%), α-pinene (11.1%), and limonene (10.8%) in *C. obtusa*; myrtenol (27.0%), myrtenyl (19.2%), and γ-cadinene (11.4%) in *C. formosensis*; β-cedrene (22.3%), δ-cadinene (17.6%), and widdrene (11.4%) in *T. cryptomerioides*; β-cedrene (26.2%) α-pinene (19.7%) and limonene (13.2%) in *C. lanceolata*; 3-carene (21.0%), *p*-cymene (11.0%), and limonene (9.5%) in *C. japonica*; and *p*-cymene (24.4%), terpinen-4-ol (16.6%), and α-terpineol (12.5%) in *C. macrolepis*. The results may provide useful information for future studies on chemotaxonomy and metabolomics of conifers.

Keywords: *Calocedrus macrolepis*; *Chamaecyparis formosensis*; *Chamaecyparis obtusa*; chemotaxonomy; conifer; *Cryptomeria japonica*; *Cunninghamia lanceolata*; fragrance; metabolomics; SPME; *Taiwania cryptomerioides*.

Introduction

Being richly endowed by nature, there are many precious tree species grown in Taiwan. Many of them are widely studied all over the world. In Taiwan, the following trees are known as “the five precious woods of Taiwan”: *Taiwania cryptomerioides*, *Calocedrus macrolepis* var. *formosana*, *Cunninghamia lanceolata*, *Chamaecyparis formosensis*, and *Chamaecyparis obtusa* var. *formosana* (Wang et al. 2005a). Their pleasant fragrance is one of the reasons for their popularity. It has been proved that fragrance directly stimulates the limbic lobe and hypothalamus, thus exerting a profound effect on the mind and body (Kodis et al. 1998). These woods not only have pleasant fragrance, but also excellent durability. Properties such as color, smell, durability, etc., are due to extractives, which are secondary metabolites (Kai 1991). In his excellent review, Imamura (1989) summarized the odorous constituents of some coniferous woods. For example, bornyl acetate, camphene, carvacrol, limonene, myrcene, α- and β-pinene, and α-terpineol contribute to the fragrance of *Chamaecyparis* spp.; the main fragrances of *Cryptomeria japonica* are δ-cadinene, δ-cadinol, β-eudesmol, and copaene. Chang et al. (1998) isolated several fragrance compounds from conifers grown in Taiwan. For example, α-cadinol was isolated from *T. cryptomerioides*, cedrol from *C. lanceolata*, and α-terpineol and α-cadinol from *C. obtusa* var. *formosana* by liquid chromatography (LC) and HPLC. It was reported that the odor of these compounds was similar to that of the woods they were isolated from. However, analysis of fragrance is still a field that requires much research. It is especially difficult to quantify the value of odors. The sensitivity of olfaction varies widely among individuals, and is also affected by environmental conditions. Most of the so-called fragrance compounds of wood were either obtained from essential oils or crude extractives, i.e., the compounds do not originate directly from wood. The true odor profile of a wood cannot be established based on traditional extraction or separation procedures.

Recently, solid-phase microextraction (SPME) has been developed for effective preparation and analysis of volatile components. SPME uses a fiber coated with a liquid (polymer), a solid (sorbent), or a combination of both. The solid fiber coating, in the case of fragrance analysis, removes the compounds from a gas sample by absorption or adsorption. The SPME fiber is then inserted directly into a GC instrument using a syringe. The volatile substances are desorbed and analyzed. Pawliszyn (1997), the inventor, applied this technique to the analysis of aromatic plants. The advantages of the technique are simplicity, speed, solvent-free, high sensitivity, small sample volume, lower cost and simple handling for the collection of volatile constituents (Rohloff and Bones 2005; Pellati et al. 2005). The technique is widely used in environmental, food, and clinical analyses; however, fewer applications are known for the study of wood fragrances. In this study, the SPME technique was used in combination with GC/MS for the characterization of precious coniferous woods of Taiwan.

Table 1 Coniferous wood samples used in this study.

Species	Tree age (years)	Locality
Chamaecyparis formosensis	80	Chitou Experimental Forestry, National Taiwan University
Chamaecyparis obtusa var. *formosana*	60–70	Dasyue Mountain
Calocedrus macrolepis var. *formosana*	40	Lien-Hua-Chie Research Center, Taiwan Forestry Research Institute
Taiwania cryptomerioides	30	Huisun Experimental Forestry, Chung-Hsing University
Cunninghamia lanceolata	30	Chitou Experimental Forestry, National Taiwan University
Cryptomeria japonica	25	Huisun Experimental Forestry, Chung-Hsing University

All samples were collected between June 2005 and July 2005.

Materials and methods

Wood samples and preparation

Table 1 lists the coniferous woods studied. Wood samples were ground into meal (40–80 mesh) then frozen at -20°C until analyzed.

Solid phase microextraction

A SPME holder and carboxen-polydimethylsiloxane-coated fibers (75 μm) were purchased from Supelco Co. (Bellefonte, USA). Before use, SPME fibers were conditioned by heating in a hot injection port of a GC at 250°C for 20 min to remove contaminants. A sample (3 g) of wood meal was placed in a 20-ml sample vial sealed with Parafilm. The vial was placed in a water bath (30$\pm$2°C) and conditioned (20 min, without fiber). After the equilibration time, the fiber was introduced into the vial and exposed to the gases in the headspace of the wood meal for 10 min. The parameters chosen are based on previous experiments (Wang et al. 2005b). After 10 min, the SPME fiber was immediately inserted into the injection port of the GC using an SPME liner for desorption at 250°C for a 30 s splitless period. The GC column temperature was maintained at 40°C.

GC/MS analyses

A HP G1800A GC/MS instrument was used with a DB-5 column (30 m$\times$0.25 mm i.d., 0.25 μm film thickness, J & W Scientific). The column temperature was held at 40°C for 1 min, then increased at 4°C min^{-1} to 260°C and held for 4 min. The injector temperature was 250°C and the ion source temperature was 280°C with EI of 70 eV. The carrier gas was He at a flow rate of 1 ml min^{-1}, and a split ratio of 1:50 was used. The MS scan range was m/z 45–425. The libraries Wiley (V. 7.0)/NBS (V. 2.0) Registry of Mass Spectral Database were searched and authentic reference compounds were used for substance identification. The GC peak areas were used for quantification without individual response factors.

Data analyses

Cluster analyses and principal components analyses (PCA) were performed with MVSP (multivariate statistical package; Koach 1999) to evaluate the similarity of fragrance compounds emitted from coniferous woods.

Results and discussion

The SPME results obtained from the six woods studied are summarized in Table 2. *C. formosensis* and *C. obtusa* (both called hinoki wood in Taiwan and Japan) are highly appreciated for their excellent quality, fragrance and durability (Wang et al. 2005a). Their odors are distinctly different. It is simple to identify these two hinoki woods according to their characteristic odor: sweet is the main characteristic note of the former and pungent of the latter. *C. obtusa* showed the highest diversity of compounds detected by SPME-GC/MS. β-Elemene (15.8%), γ-cadinene (12.1%), α-pinene (11.1%), and limonene (10.8%) are the most abundant compounds in this wood. As Table 2 reveals, the main compounds in *C. formosensis* are myrtenol (27.0%), myrtenyl (19.2%), and γ-cadinene (11.4%). According to our previous results for the essential oil of *C. formosensis* (Wang et al. 2005a), α-eudesmol (18.0%), myrtenol (14.1%) and myrtenyl (1.9%) should be the major compounds. α-Eudesmol, however, was not detected by SPME among the volatile compounds of the wood. Based on the results obtained in this study, we suggest that myrtenol and myrtenyl may contribute to the sweet smell of *C. formosensis* wood, and a mixture of monoterpenes, such as α-pinene, limonene, α-terpinolene, and α-terpineol, may cause the pungent odor of *C. obtusa*.

C. macrolepis (Cupressaceae), with the common name incense-cedar, was also studied. It has been used as a raw material for incense since ancient times in Taiwan. Cheng et al. (2004) analyzed the leaf essential oil of *C. macrolepis* and found α-pinene (44.2%), limonene (21.6%), β-myrcene (8.9%), β-caryophyllene (8.2%), caryophyllene oxide (2.4%), α-cadinol (1.6%), β-pinene (1.2%), and T-muurolol (1.1%). The essential oil had strong anti-termite and antifungal properties. However, there has been no study of the fragrance of *C. macrolepis* wood itself. Table 2 reveals that *C. macrolepis* emits mainly the volatiles *p*-cymene (24.4%), terpinen-4-ol (16.6%), and α-terpineol (12.5%).

The results for *T. cryptomerioides*, *C. lanceolata*, and *C. japonica* (all three trees belong to the Taxodiaceae) are also presented in Table 2. *C. lanceolata* contains α-pinene (19.7%) and limonene (13.2%). In this regard it is similar to the composition of the monoterpene fraction for *C. obtusa* (Cupressaceae). However, the most abundant fragrance compound in *C. lanceolata* is β-cedrene (26.2%), which is absent in *C. obtusa*. *T. cryptomerioides* is one of the most representative conifers native in Taiwan. According to the overviews published by Wang et al. (1997), Kuo et al. (1999), and Chang et al. (2003), more than 200 secondary metabolites have been identified from *T. cryptomerioides*. Many studies had focused on the bioactivity of chemical compounds from *T. crypto-*

Table 2 GC/MS results for fragrance compounds from six coniferous woods grown in Taiwan.

	KI[a]	Concentration (%)						Identification[b]
		CO	CF	CM	CL	TC	CJ	
α-Thujene	921	–	–	–	–	–	0.9	MS, KI
α-Pinene	929	11.1	1.3	–	19.7	–	6.1	MS, KI, ST
α-Fenchene	951	–	–	2.4	–	–	–	MS, KI
Camphene	953	0.5	–	–	0.8	–	1.3	MS, KI, ST
Sabinene	967	–	–	–	–	–	4.7	MS, KI
Myrcene	983	0.2	–	–	2.7	–	9.4	MS, KI, ST
α-Phellandrene	1005	–	–	0.3	–	–	–	MS, KI, ST
3-Carene	1006	–	–	–	0.1	–	21.0	MS, KI, ST
α-Terpinene	1010	–	–	5.6	–	–	–	MS, KI, ST
p-Cymene	1016	–	–	24.4	–	–	11.0	MS, KI, ST
Limonene	1023	10.8	0.2	9.4	13.2	–	9.5	MS, KI, ST
γ-Terpinene	1052	0.1	–	0.9		–	9.1	MS, KI, ST
Terpinolene	1082	3.8	–	–	0.7	–	5.7	MS, KI, ST
Linalool	1085	–	–	0.3	–	–	0.3	MS, KI, ST
endo-Fenchol	1112	1.5	–		0.8	–	–	MS, KI
Camphor	1143	0.5	–	2.4	0.1	–	–	MS, KI, ST
Borneol	1165	3.0	–	–	1.3	–	–	MS, KI
Terpinen-4-ol	1174	–	–	16.6	–	–	8.0	MS, KI, ST
α-Terpineol	1183	2.2	–	12.5	2.7	–	0.3	MS, KI, ST
Myrtenol	1194	–	27.0	–	–	–	–	MS, KI
Verbenone	1204	–	–	3.9	–	–	–	MS, KI
exo-2-Hydroxy-1,4-cineole	1219	–	–	0.4	–	–	–	MS, KI
Piperitone	1226	–	–	1.3	–	–	–	MS, KI
Myrtenyl	1233	–	19.2	–	–	–	–	MS, KI
Bornyl acetate	1278	0.2	2.2	–	0.5	–	1.1	MS, KI, ST
α-Terpinyl acetate	1339	0.5	–	–	0.7	–	8.6	MS, KI
α-Cubebene	1345	–	2.0	–	–	0.1	–	MS, KI
α-Copaene	1376	2.6	1.7	–	–	1.7	–	MS, KI
β-Elemene	1393	15.8	3.6	–	–	–	–	MS, KI
α-Cedrene	1409	–	–	1.0	9.6	5.5	–	MS, KI
β-Cedrene	1418	–	–	–	26.2	22.3	–	MS, KI
Widdrene	1431	–	–	–	4.2	11.4	–	MS, KI
(*E*)-β-Farnesene	1459	–	–	0.5	3.9	6.8	–	MS, KI
α-Humulene	1463	0.3	–	–	–	1.5	–	MS, KI
α-Amorphene	1485	9.4	6.3	–	–	5.4	–	MS, KI
β-Selinene	1485	5.0	4.1	–	0.4	–	–	MS, KI
α-Selinene	1493	2.2	0.6	–	–	–	–	MS, KI
α-Muurolene	1499	3.9	5.3	–	–	11.2	–	MS, KI
Cuparene	1502	–	–	–	1.1	1.0	–	MS, KI
γ-Cadinene	1514	12.1	11.4	–	–	8.9	–	MS, KI
δ-Cadinene	1524	9.0	4.3	–	–	17.6	–	MS, KI
Cadina-1,4-diene	1532	–	–	–	–	0.2	–	MS, KI
Cedrol	1596	–	–	–	4.9	0.4	–	MS, KI, ST
T-Muurolol	1635	0.5	0.6	–	–	–	–	MS, KI
δ-Cadinol	1646	–	–	–	–	0.4	–	MS, KI
α-Cadinol	1654	0.3	0.4	–	–	0.3	–	MS, KI

CO, *C. obtusa*; CF, *C. formosensis*; CM, *C. macrolepis*; CL, *C. lanceolata*; TC, *T. cryptomeri*; CJ, *C. japonica*; KI, Kovats index; ST, authentic standard compounds; – not detected.
[a]Kovats index on a DB-5 column in reference to *n*-alkanes.
[b]MS, NIST and Wiley libraries and the literature.

merioides (Wang et al. 2002; Chang et al. 2003). Concerning the fragrance in *T. cryptomerioides* wood, Chang et al. (2003) demonstrated that the odor of α-cadinol, T-cadinol, and T-muurolol, which were isolated from methanol extractives of *T. cryptomerioides* wood, are similar to the odor of the *T. cryptomerioides* wood itself. The main volatile constituents of *T. cryptomerioides* are β-cedrene (22.3%), δ-cadinene (17.6%), and α-muurolene (11.2%). It is remarkable that *T. cryptomerioides* lacks monoterpenes, such as α-pinene, limonene, α-terpineol, and terpinen-4-ol, which are common in other woods.

Although *C. japonica* is not an endemic tree species of Taiwan, it has become economically very important over the past decades. According to Imamura (1989), the main fragrances of *C. japonica* are δ-cadinene, δ-cadinol, β-eudesmol, and copaene. We identified 3-carene (21.0%), *p*-cymene (11.0%), and limonene (9.5%) by SPME-GC/MS.

Figure 1 illustrates the main findings of the present work. Concerning the chemical skeleton of the fragrances identified, the essential compounds in *C. obtusa* belong to the cadinane-type and 2,6-dimethyloctane-

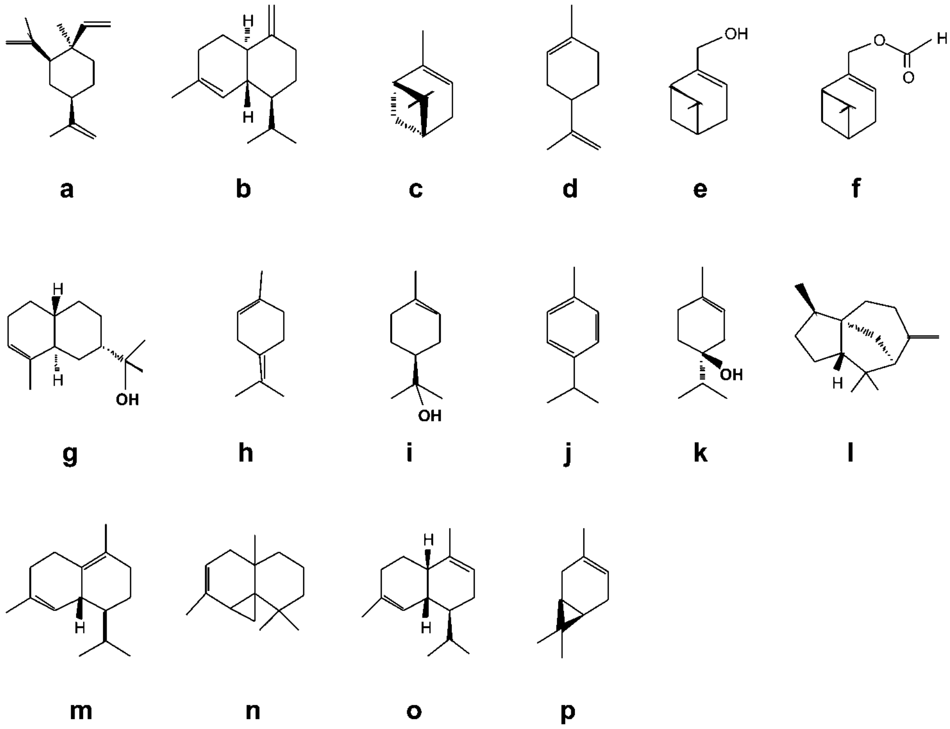

Figure 1 Main fragrance compounds of six coniferous woods. (a) β-Elemene, (b) γ-cadinene, (c) α-pinene, (d) limonene, (e) myrtenol, (f) myrtenyl, (g) α-eudesmol, (h) terpinolene, (i) α-terpineol, (j) *p*-cymene, (k) terpinen-4-ol, (l) β-cedrene, (m) δ-cadinene, (n) widdrene, (o) α-muurolene, and (p) 3-carene.

type terpenoids. The dominant skeleton in *C. formosensis* belongs to the pinane type. The menthane-type monoterpenes are abundant in *C. macroplepis*. In the Taxodiaceae family, the most abundant skeleton found in *T. cryptomerioides* is of the cedrane and cadinane type; in *C. lanceolata*, the cedrane and pinane type; and in *C. japonica*, the carane and menthane type.

Principal component analysis (PCA) and cluster analysis were performed to detect the degrees of similarity of the compositions of the woods analyzed (Figure 2). Three different groups can be identified in the loading plots of PCA 1 and PCA 2, namely: (1) *C. formosensis* and *C. obtusa*; (2) *T. cryptomerioides* and *C. lenceolata*; and (3) *C. japonica* and *C. macrolepis*. Moreover, the similarity of the volatile secondary metabolites can be quantified: between *C. formosensis* and *C. obtusa* there is a similarity of 42%, and between *T. cryptomerioides* and *C. lenceolata* this value is 38%. The similarity between these two groups is 28%. Although there is 30% similarity between *C. japonica* and *C. macrolepis*, they are far away from the other two groups, with a similarity of only 22%.

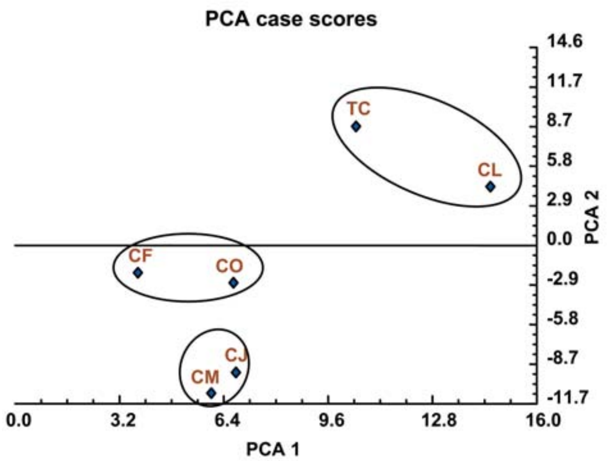

Figure 2 Results of PCA analysis for fragrance secondary metabolites of six conifers.

Conclusions

SPME coupled to GC/MS is well suited for elucidation of the fragrance composition of coniferous woods (Figure 3). The technique is quick and powerful. In the post-genomic era, metabolomics studies (i.e., systematic studies of the unique chemical fingerprints that specific

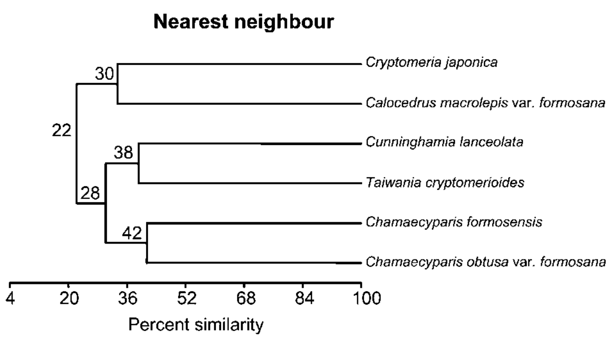

Figure 3 Similarity analysis of fragrance compound compositions from six coniferous woods.

cellular processes leave behind) will become as important as functional genomics and proteomics. SPME-GC/MS may contribute much to this research area.

References

Chang, S.T., Wang, S.Y., Wu, C.L., Chang, H.T. (1998) Isolation and identification of fragrant constituents from three coniferous woods grown in Taiwan (in Chinese with English abstract). For. Prod. Ind. 17:737–750.

Chang, S.T., Wang, S.Y., Kuo, Y.H. (2003) Resources and bioactive substances from Taiwania (*Taiwania cryptomerioides*). J. Wood Sci. 49:1–4.

Cheng, S.S., Wu, C.L., Chang, H.T., Kao, Y.T., Chang, S.T. (2004) Antitermitic and antifungal activity of essential oil of *Calocedus formosana* leave and its composition. J. Chin. Ecol. 30:1957–1967.

Imamura, H. (1989) Contribution of extractives to wood characteristic. In: Natural Products of Woody Plants. Ed. Timell, T.E. Springer-Verlag, Berlin. pp. 851–856.

Kai, Y. (1991) Chemistry of extractives. In: Wood and Cellulosic Chemistry. Eds. Hon, D.N.S. Shiraishi, N. Marcell Dekker, New York. pp. 215–255.

Koach (1999) MVSP – A multivariate statistical package for Windows, version 3.1. Koach Computing Services, Pentraeth, UK.

Kodis, M., Moran, D.T., Houy, D. Love Scents. EP Dutton, New York, 1998. 212 pp.

Kuo, Y.H., Yu, M.T., Li, Y.C., Shiu, L.L. (1999) Natural products research in Taiwan (V). Formosan Science 52:1–145.

Pawliszyn, J. (1997) Operating principles and construction of SPME devices. In: Solid Phase Microextraction Theory and Practice. Wiley-VCH, New York. pp. 11–42.

Pellati, F., Benvenuti, S., Yoshizaki, F., Bertelli, D., Rossi, M.C. (2005) Headspace solid-phase microextraction-gas chromatography–mass spectrometry analysis of the volatile compounds of *Evodia* species fruits. J. Chromatogr. A 1087: 265–273.

Rohloff, J., Bones, A.M. (2005) Volatile profiling of *Arabidosis thaliana* – putative olfactory compounds in plant communication. Phytochemistry 66:1941–1955.

Wang, S.Y., Chang, S.T., Su, Y.C., Kuo, Y.H. (1997) Studies on the extractives of Taiwania (*Taiwania cryptomerioides* Hayata): A review (in Chinese with English abstract). Q. J. Exp. For. Natl. Taiwan Univ. 11:67–81.

Wang, S.Y., Wu, J.H., Shyur, L.F., Kuo, Y.H., Chang, S.T. (2002) Antioxidant activity of abietane-type diterpenes from heartwood of *Taiwania cryptomerioides* Hayata. Holzforschung 56:487–492.

Wang, S.Y., Wu, C.L., Chu, F.H., Chien, S.C., Kuo, Y.H., Shyur, L.F., Chang, S.T. (2005a) Chemical composition and antifungal activity of essential oil isolated from *Chamaecyparis formosensis* Matsum. wood. Holzforschung 59:295–299.

Wang, Y.S., Lin, C.T., Liu, C.P., Wang, S.Y. (2005b) Characterization and analysis of fragrance constituents of precious coniferous wood (in Chinese with English abstract). In: Proceedings of the Annual Meeting of the Chinese Forestry Association, October 2005, Pingtong, Taiwan. pp. 84–91.

Received March 9, 2006. Accepted June 2, 2006.