Holzforschung
54 (2000) 241–245

Comparison of the Antifungal Activity of Cadinane Skeletal Sesquiterpenoids from Taiwania (*Taiwania cryptomerioides* Hayata) Heartwood

By Shang-Tzen Chang[1], Sheng-Yang Wang[1], Chi-Lin Wu[1], Pin-Fun Chen[1] and Yueh-Hsiung Kuo[2]

[1] Department of Forestry, National Taiwan University, Taipei, Taiwan
[2] Department of Chemistry, National Taiwan University, Taipei, Taiwan

Keywords

Taiwania cryptomerioides Hayata
Antifungal compound
α-Cadinol
T-Muurolol
T-Cadinol

Summary

The antifungal activity of cadinane skeletal sesquiterpenoids from Taiwania (*Taiwania cryptomerioides* Hayata) heartwood is demonstrated. Using spectral analyses, the absolute structures of three main cadinanes, T-cadinol, T-muurolol, and α-cadinol, all isolated from Taiwania with HPLC, were identified. The amount of these cadinanes was also quantified using GC. The results showed that the total amount of cadinanes extracted from heartwood with *n*-C_6H_{14} was 6.49 mg per kg of wood. This was much more than the essential oils collected by water distillation from leaves (0.04 mg/kg), sapwood (0.36 mg/kg), or heartwood (1.77 mg/kg). Moreover, results obtained from the antifungal assays demonstrated that α-cadinol exhibited the highest antifungal index for both *Coriolus versicolor* and *Laetiporus sulphureus*, followed by T-cadinol and T-muurolol. As a matter of fact, α-cadinol completely inhibited the growth of *C. versicolor* and *L. sulphureus* at the level as low as 100 ppm. Further comparison of the molecular configuration of these cadinanes reveals that cadinane skeletal sesquiterpenoids with an equatorial hydroxyl group at C-9 and a *trans* configuration at the ring junction, such as the case for α-cadinol, exhibited the strongest antifungal activity.

Introduction

Developing methods to prolong the service life of wood has always been the interest of wood industry researchers. From the environmental perspective, finding naturally existent constituents in highly durable species and elucidating the mechanisms of them are the most appropriate approaches to achieve wood protection while preserving the environment. Taiwania (*Taiwania cryptomerioides* Hayata) is an endemic tree that grows on elevations from 1800 to 2600 meters in Taiwan's central mountains. The heartwood of Taiwania is yellowish red with distinguished purplish pink streaks. In addition, Taiwania is extremely decay resistant.

We have conducted several studies to understand the effects of chemical constituents to the wood properties of Taiwania. A collection of known lignans was first isolated from Taiwania heartwood (Wang *et al.* 1998; Su *et al.* 1998; Chang *et al.* 1999a). One of the color substances, taiwanin A with a diene structure, was also isolated from Taiwania heartwood. It was further confirmed by spectral analyses to be of a *trans-trans* formulation (Chang *et al.* 1999b). Taiwanin A, a deep orange crystalline, turned into whitish and light yellow compounds of taiwanin C and taiwanin E, respectively, after exposing to light irradiation (Chang *et al.* 1999b). Other compounds including α-cadinol, α-cedrol, hinokiol, sugiol, ferruginol, helioxanthin, savinin, and taiwanin C that were isolated from Taiwania heartwood in our previous work were also studied for their antifungal effectiveness (Chang *et al.* 1998, 1999c). Among all these, α-cadinol has demonstrated to possess the highest antifungal effectiveness. Kondo and Imamura had also investigated the antifungal compounds in heartwood extractives of *Chamaecyparis obtusa* (Kondo and Imamura 1986). Using gas-liquid chromatography analysis, they deduced that the main antifungal compounds of *Chamaecyparis obtusa* were cadinane skeletal sesquiterpenoids. Other literature (Wang *et al.* 1997) stated that in addition to α-cadinol, there are other cadinane skeletal sesquiterpenoids, such as T-cadinol and T-muurolol presented in Taiwania. Unfortunately, it is beyond its capability to isolate and purify these compounds with open column chromatography (CC) or thin layer chromatography (TLC). Therefore, further comparison of the antifungal effectiviness of these cadinane skeletal sesquiterpenoids has not been available. In this study, the cadinane skeletal sesquiterpenoids were successfully isolated and purified from Taiwania heartwood. The amounts of these cadinane skeletal sesquiterpenoids and their antifungal activity were also examined.

Materials and Methods

Instruments

HPLC (High Performance Liquid Chromatography) was performed using a Jasco model PU980 pump equipped with a Jasco RI-930 RI detector and Hibar Lichrosorb Si 60 (25×1 cm i.d.) column. The IR spectra were recorded on a Bio-Rad model FTS-40

spectrophotometer and the MS was obtained using a Finnigan MAT-958 Mass spectrometer. The ^{13}C and ^{1}H NMR spectra were recorded on a Bruker Avance- 300 MHz FT-NMR.

Extraction and isolation

A twenty-seven-year-old Taiwania (*Taiwania cryptomerioides* Hayata) tree was selected and cut in the Experimental Forest of National Taiwan University. Heartwood chips were prepared from the freshly cut tree. The essential oils from various parts of this tree including leaves, sapwood, and heartwood were collected using water distillation. Air-dried heartwood chips weighing 5.7 kg were exhaustively extracted with methanol (MeOH). The extractives were condensed to *ca* 286.4 g, followed by a chain of extraction with *n*-hexane (*n*-C_6H_{14}), chloroform ($CHCl_3$), ethyl acetate (EtOAc), and methanol (MeOH). The solvents of the successive extraction, *n*-C_6H_{14}, $CHCl_3$, EtOAc, and MeOH were then removed and the soluble fractions and MeOH insoluble fraction were obtained. Chromatograph was then utilized. It was equipped with a silica-gel column eluted with EtOAc/*n*-C_6H_{14} (gradient elution was performed by changing from 0/100 to 100/0), the *n*-C_6H_{14} soluble fraction (5000 mg) was divided into 41 subfractions (H1-H41). Compounds **1** to **4**, as shown in Figure 1, were isolated and purified from H16 to H22 by semi-preparative HPLC (Column: Si-60 column, Mobil phase: EtOAc/*n*-C_6H_{14} = 30/70, Flow rate: 1 ml/min).

GC analysis

GC was performed using a Shimadzu model-14B equipped with a FID. The column used was 50 m long by 0.22 mm i.d. glass capillary coated with silica. GC was programmed from 60 to 220 °C at 2 °C/min. The quantity of compounds was obtained by integrating the peak area of spectrograms.

Antifungal assays

Fungi used in this study were *Coriolus versicolor* (L. ex Fr.) Quel. and *Laetiporus sulphureus* (B. ex Fr.) Bond.. Antifungal assays were performed three times, and the data were averaged. One hundred ppm cadinane skeletal sesquiterpenoids (compounds **1, 2** and **4** as shown in Fig. 1) isolated from H16 to H22 were added to sterilized potato dextrose agar (PDA). After transferring the mycelium of *C.v.* and *L. s.*, the testing plates were incubated at 27 °C. When the mycelium of fungi reached the edges of the control plate (without adding extractives), the antifungal index was calculated as follows:

Antifungal index = (1− Da/Db) ×100
Da: radial growth mycelium on the testing plate.
Db: the diameter of testing plate.

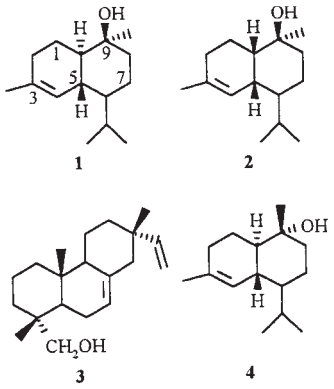

Fig. 1. Configurations of T-cadinol (**1**), T-muurolol (**2**), isopimarinol (**3**), and α-cadinol (**4**) isolated from Taiwania.

*Compound **1** (T-cadinol)*

Yellow oil, EIMS for $C_{15}H_{26}O$ (EIMS: 222), ^{1}H-NMR (in $CDCl_3$) : δ(ppm) 0.76 (d, *J* = 7.0, H-12), 0.88 (d, *J* = 7.0, H-13), 1.19 (s, H-14), 1.70 (s, H-15), 2.16 (m, H-11) 5.52 (s, H-4). ^{13}C-NMR : δ(ppm) 15.18 (C-12), 19.79 (C-7), 21.40 (C-13), 22.57 (C-1), 23.76 (C-15), 26.15 (C-11), 28.44 (C-14), 30.88 (C-2), 37.71 (C-5), 40.28 (C-8), 46.64 (C-6), 47.91 (C-10), 70.69 (C-9), 122.63 (C-4), 134.35 (C-3).

*Compound **2** (T-muurolol)*

Colorless crystal, m.p. 80-81, EIMS for $C_{15}H_{26}O$ (EIMS: 222), ^{1}H-NMR (in $CDCl_3$) : δ(ppm) 0.80 (d, *J* = 7.0, H-12), 0.86 (d, *J* = 7.0, H-13), 1.17 (s, H-14), 1.62 (s, H-15), 2.24 (m, H-11) 5.53 (d, *J* = 7.0, H-4). ^{13}C-NMR : δ(ppm) 15.36 (C-12), 19.35 (C-7), 20.92 (C-13), 21.55 (C-1), 23.57 (C-15), 26.64 (C-11), 29.24 (C-14), 31.24 (C-2), 34.49 (C-5), 34.62 (C-8), 43.94 (C-6), 46.09 (C-10), 72.34 (C-9), 124.84 (C-4), 133.45 (C-3).

*Compound **3** (isopimarinol)*

Gum, EIMS for $C_{20}H_{32}O$ (EIMS:288), IR νmax : 3350, 1635, 995, 910 cm^{-1}, ^{1}H-NMR (in $CDCl_3$) : δ(ppm) 0.79 (s, H-19), 0.82 (s, H-20), 1.02 (s, H-17), 3.10 (d, *J* = 10.1, H-18), 3.37 (d, *J* = 10.1, H-18), 4.84 (dd, *J* = 17.5, 1.2 Hz, H-16), 4.90 (dd, *J* = 17.5, 1.2 Hz, H-16), 5.19 (br, H-7), 5.76 (dd, *J* = 17.5, 10.8, H-15). ^{13}C-NMR (in $CDCl_3$) : δ(ppm) 15.6 (C-20), 17.9 (C-2), 18.3 (C-19), 18.8 (C-11), 22.4 (C-17), 25.9 (C-6), 34.5 (C-10), 35.4 (C-3), 35.7 (C-12), 37.4 (C-13), 37.8 (C-4), 38.1 (C-1), 38.7 (C-5), 47.9 (C-14), 50.5 (C-9), 72.2 (C-18), 110.0 (C-16), 128.7 (C-7), 137.0(C-8), 149.1 (C-15).

*Compound **4** (α-cadinol)*

Colorless needle crystal, m.p. 74-75 °C, EIMS for $C_{15}H_{26}O$ (EIMS: 222), ^{1}H-NMR (in $CDCl_3$) : δ(ppm) 0.74 (d, *J* = 7.0, H-12), 0.89 (d, *J* = 7.0, H-13), 1.08 (s, H-14), 1.64 (s, H-15), 2.13 (m, H-11) 5.47 (s, H-14). ^{13}C-NMR : δ(ppm) 15.13 (C-12), 20.77 (C-14), 21.51 (C-13), 22.00 (C-1), 22.68 (C-7), 23.81 (C-15), 26.00 (C-11), 30.95 (C-2), 39.89 (C-5), 42.22 (C-6), 46.73 (C-6), 50.04 (C-10), 72.47 (C-9), 122.35 (C-4), 134.94 (C-3).

Results and Discussion

Taiwania is one of the economically important tree species indigenous to Taiwan. So far we have conducted several studies on the extractives of Taiwania to understand their functions on wood properties (Wang *et al*. 1994; Chang *et al*. 1996; Wang *et al.* 1998; Su *et al.* 1998; Chang *et al.* 1998, 1999a, 1999b, 1999c). Because of its excellent decay resistant characteristics, the identification of the antifungal compounds in Taiwania heartwood has been one of the most important tasks in our continued studies. Although several constituents isolated from Taiwania have proven antifungal effectiveness, α-cadinol has by far exhibited the strongest fungal growth inhibition property (Chang *et al.* 1998, 1999c). There has been several reports stating that α-cadinol exhibites significant effectiveness in durable tree species against fungi or termites (Kondo and Imamura 1986; Kinjo *et al*. 1988; Chang and Wang 1995). Furthermore, He and his coworkers revealed that α-cadinol possessed an antitumor property (He *et al*. 1997). In one of our previous papers, we explained that how the α-cadinol was isolated from the *n*-C_6H_{14} soluble fraction of Taiwania heartwood with open column chromatography (Chang *et al.* 1998). In this study, we took a further step to isolate sever-

al cadinane skeletal sesquiterpenoids, in addition to α-cadinol, from Taiwania. The fractions (H16 to H22) that eluted from the same polarity range (EtOAc/ *n*-C_6H_{14} = 15/85) of eluting solvents were then further isolated using a HPLC.

After careful separation, fractions combined from H16 to H22 were further isolated to give compound **1** (retention time (R.t.) = 22.1 min), compound **2** (R.t. = 24.0 min), compound **3** (R.t. = 27.6 min), and compound **4** (R.t. = 31.0 min) using a semi-preparative HPLC system (column: Si-60 column, mobil phase: EtOAc/*n*-C_6H_{14} = 30/70, flow rate: 1 ml/min). M.p., MS, ^{1}H-NMR, and ^{13}C-NMR spectra were then used to identify these compounds. According to the results of spectral analyses and comparison with data from literatures, compounds **1**, **2**, **3**, and **4** (Fig. 1) were identified as T-cadinol, T-muurolol (Cheng *et al*. 1967, 1968), isopimarinol (Wenkert and Buckwalter 1972), and α-cadinol (Cheng *et al*. 1966; Chang *et al*. 1998), respectively. With the exception that isopimarinol was a diterpenoid, others were cadinane skeletal sesquiterpenoids which had also been isolated from essential oils of Taiwania heartwood by Cheng and coworkers. Although isopimarinol was a diterpenoid commonly found in coniferous trees, this is the first time that it is isolated from Taiwania as demonstrated in this study.

To quantify the cadinane skeletal sesquiterpenoids distribution in different parts of Taiwania, GC was applied to analyze the *n*-C_6H_{14} extractives and essential oils collected by water distillation from various parts of Taiwania. Table 1 shows the amounts of *n*-C_6H_{14} extractives and essential oils distilled from various parts of Taiwania. The results indicated that the amounts of essential oils ranged from 0.19 to 2.64 ml/kg. The heartwood contained the most abundance of essential oils (2.64 ml/kg), followed by leaves (0.70 ml/kg), and then sapwood (0.19 ml/kg). On the other hand, the amount of *n*-C_6H_{14} extractives was found to be 2.36 % based on wood weight. Figure 2 exhibits the GC chromatogram of essential oils that were distilled from Taiwania heartwood. By comparing with the R.t. of standards, the structures of peaks **1**, **2**, and **3,** as shown in Figure 2, were identified. They are T-cadinol, T-muurolol, and α-cadinol, respectively. After integrating the peak areas of the GC chromatograms of T-cadinol, T-muurolol, and α-cadinol from various parts of Taiwania, the amounts of these cadinanes were quantified. Table 2 shows the relative amounts of cadinanes in various parts of Taiwania. Excluding the leaves' essential oils, the amount of cadinane contents was highest in α-cadinol, followed by T-muurolol, and then T-cadinol. The cadinanes were the main constituents in heartwood essential oils (66.7 %). In leaves, however, the relative content of T-cadinol (3.3 %) was higher than that of α-cadinol (1.6 %) and T-muurolol (1.2 %). The total contents of cadinanes were only 6.1 % in leaves' essential oils. After converting the relative contents to the real contents in different parts of Taiwania, the total amount of cadinanes in *n*-C_6H_{14} extractives of 6.49 mg per kg of wood was much higher than the essential oils that were collected from leaves (0.04 mg/kg), sapwood (0.36 mg/kg), or heartwood (1.77 mg/kg) (Fig. 3).

Table 1. The amounts of hexane extractives and essential oils distilled from various parts of Taiwania

Extracts	Amounts (ml/kg)
Essential oils, leaves	0.70
Essential oils, sapwood	0.19
Essential oils, heartwood	2.64
Hexane extractives, heartwood	2.36*

*: % (based on wood weight)

Table 2. The relative contents (%) of cadinanes in various parts of Taiwania

Extracts	T-Cadinol	T-Muurolol	α-Cadinol	Total cadinane
Essential oils, leaves	3.3	1.2	1.6	6.1
Essential oils, sapwood	8.0	11.7	17.4	37.1
Essential oils, heartwood	12.8	17.1	36.8	66.7
Hexane extractives, heartwood	5.0	7.6	14.8	27.4

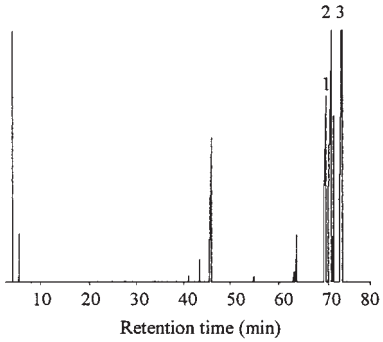

Fig. 2. GC chromatograms of essential oils that were distilled from Taiwania heartwood. (1) T-cadinol, (2) T-muurolol, and (3) α-cadinol.

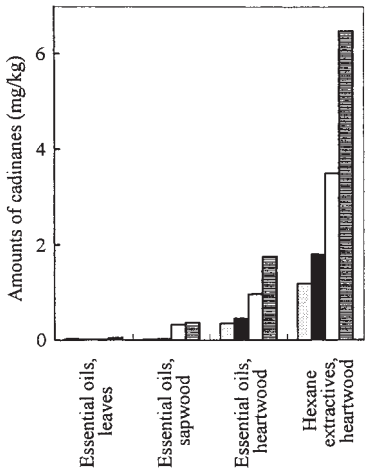

Fig. 3. The amounts of cadinanes in various parts of Taiwania. ⬚ = T-cadinol, ■ = T-muurolol, □ = α-cadinol, ▒ = total cadinane.

In order to illustrate the antifungal effectiveness of cadinanes isolated from Taiwania, we selected *Coriolus versicolor* (white rot fungi) and *Laetiporus sulphureus* (brown rot fungi) for antifungal assay. Both *C. versicolor* and *L. sulphureus* were commonly found in the decayed wood in Taiwan. The antifungal indices of T-cadinol, T-muurolol, and α-cadinol against *C. versicolor* and *L. sulphureus* were presented in Table 3. Based on the results of antifungal test, the order of antifungal effectiveness of three cadinanes for *C. versicolor* was α-cadinol (100.0) as the best, and then T-cadinol (47.1) and T-muurolol (38.8). For *L. sulphureus* α-cadinol (100.0) was again the best, but equal by T-cadinol (100.0), and followed by T-muurolol (82.0). Although the structures of these three compounds were similar, they demonstrated different antifungal effectiveness. Among them, α-cadinol was the strongest antifungal compound. It completely inhibits *C. versicolor* and *L. sulphureus* growth at the level of 100 ppm. The antifungal activity of T-cadinol and T-muurolol was not as effective as α-cadinol, especially against *C. versicolor.* Nevertheless, they both exhibited good antifungal effectiveness to *L. sulphureus.* Correlating the antifungal effectiveness and molecular configurations of cadinanes indicates that whatever against *C. versicolor* or *L. sulphureus*, an equatorial hydroxyl group at C-9 and a *trans* configuration at ring junction (C-5 connects to C-10; α-cadinol) exist. It was also noticed that the stereo configuration of hydroxyl at C-9 was less important than the configuration at ring junction for the growth inhibition of *L. sulphureus*. Both α-cadinol and T-cadinol have *trans* configuration at the ring junction and as a result they completely inhibit the mycelial growth of fungi. As for T-muurolol, the least effective antifungal one among these cadinanes, has a *cis* configuration and an axial hydroxyl group at C-9.

Table 3. Antifungal indices of T-cadinol, T-muurolol, and α-cadinol (100 ppm)

Fungi	T-Cadinol	T-Muurolol	α-Cadinol
C.versicolor	47.1	38.8	100
L. sulphureus	100	82.0	100

Conclusions

Cadinane skeletal sesquiterpenoids are the dominant compounds in Taiwania. They contribute to the antifungal effectiveness of heartwood. The main cadinanes in Taiwania including T-cadinol, T-muurolol, and α-cadinol were extracted using HPLC in this study. The total amount of these cadinanes was also quantified using GC. The results showed that the total amount of cadinanes extracted from heartwood with *n*-C_6H_{14} (6.49 mg/kg, based on wood weight) was much more than that in the essential oils collected by water distillation from leaves (0.04 mg/kg), sapwood (0.36 mg/kg), or heartwood (1.77 mg/kg). The antifungal effectiveness of these cadinanes was also compared and the results show that an equatorial hydroxyl group at C-9 and a *trans* configuration at ring junction (C-5 connects to C-10) (α-cadinol) provide superior effectiveness against *C. versicolor* or *L. sulphureus*. Also, the stereo configuration of hydroxyl at C-9 was less important than the configuration at ring junction for the growth inhibition of *L. sulphureus*. Both α-cadinol and T-cadinol were *trans* configuration at the ring junction. They could completely inhibit the mycelial growth of fungi. Because of the least antifungal property of T-muurolol it is evident that the cadinane skeletal sesquiterpenoids with a *cis* configuration and an axial hydroxyl group at C-9 has the lowest antifungal activity.

Acknowledgements

The authors wish to thank Prof. David N. S. Hon (Clemson University) for his kind suggestions as well as Mr. H.-Y. Lin and Mr. T.-Y. Hsieh (The Experimental Forest of National Taiwan University) for providing Taiwania lumbers. This study was also supported by a grant from the National Science Council of R. O. C. and the authors would like to thank the National Science Council for this support.

References

Chang, S.-T. and S.-Y. Wang. 1995. Introduction of the termiticidal wood extractives. For. Prod. Industries. *14* (2), 149–159.

Chang, S.-T., S.-Y. Wang and Y-.C. Su. 1996. Susceptibility of the extractives to color change in Taiwania (*Taiwania cryptomerioides*) heartwood: preliminary study. Q. J. Chin. For. *29* (4), 109–124.

Chang, S.-T., C.-L. Wu, S.-Y. Wang, Y.-C. Su and Y.-H. Kuo. 1998. Studies on the antifungal compounds in the heartwood extractives of Taiwania (*Taiwania cryptomerioides* Hayata) (I): Isolation and identification of antifungal compounds in hexane soluble fraction. For. Prod. Industries. *17* (2), 287–304.

Chang, S.-T., S.-Y. Wang and Y.-C. Su. 1999a. Structural elucidation of three dibenzyl-γ-butyrolactone type lignans isolated from Taiwania (*Taiwania cryptomerioides* Hayata) heartwood: 7-Oxohinokinin, Sventenin and Arctigenin. Q. J. Chin. For. *32* (1), 121–129.

Chang, S.-T., S-.Y. Wang, Y-C. Su and Y.-H. Kuo. 1999b. Chemical constituents and mechanisms of discoloration of Taiwania (*Taiwania cryptomerioides* Hayata) heartwood—(I) The structure reconfirmation and conversion mechanism of Taiwanin A. Holzforschung *53*, 142–146.

Chang, S.-T., S.-Y. Wang, C.-L. Wu, Y.-C. Su and Y.-H. Kuo. 1999c. Antifungal compounds in the ethyl acetate soluble fraction of the extractives of Taiwania *(Taiwania cryptomerioides* Hayata*)* heartwood. Holzforschung *53* (5), 487–490.

Cheng, Y.-S., Y.-H. Kuo and Y.-T. Lin. 1966. Extractive components from the wood of *Taiwania cryptomerioides* Hayata IV. Study of essential oil. Chemistry *1*, 38–41

Cheng, Y.-S., Y-.H. Kuo and Y.-T. Lin. 1967. Extractives compounds from *Taiwania cryptomerioides* Hayata: the structures of t-cadinol and t-muurolol. J. Chem. Soc. Chem. Commun. *25*, 565–566.

Cheng, Y.-S., Y.-H. Kuo and Y.-T. Lin. 1968. Extractive components from the wood of *Taiwania cryptomerioides* Hayata V. Study of essential oil. Chemistry *1*, 47–51

He, K., L. Zeng, G. Shi, G.-X. Zhao, J. F. Kozlowski and J. L. McLaughlin. 1997. Bioactive compounds from *Taiwania cryptomerioides* Hayata. J. Nat. Prod. *60*, 38–40.

Kinjo, K., Y. Doufuku and S. Yaga. 1988. Termiticidal substances from the wood of *Chamaecyparis obtusa*. Mokuzai Gakkaishi. *34*(5), 451–455.

Kondo, R. and H. Imamura. 1986. Antifungal compounds in heartwood extractives of hinoki (*Chamaecyparis obtusa* Endl.). Mokuzai Gakkaishi *32*(3), 213–217.

Su, Y.-C., S.-T. Chang, S.-Y. Wang, C.-L. Wu, S.-L. Huang and Y.-H. Kuo. 1998. Structure elucidation of three lignans isolated from Taiwania (*Taiwania cryptomerioides* Hayata) heartwood: Savinin, Taiwanin C, and Dimethylmatiresinol. Q. J. Chin. For. *31* (3), 287–295

Wang, S.-Y., S.-T. Chang and Y.-C. Su. 1994. The effect of environmental factors on the discoloration of *Taiwania Cryptomerioides* heartwood. For. Prod. Industries. *13* (3), 351–361.

Wang, S.-Y., S.-T. Chang, Y.-C. Su and Y.-H. Kuo. 1997. Studies on the extractives of Taiwania (*Taiwania cryptomerioides* Hayata): a review. Quarterly Journal of the Experimental Forestry on National Taiwan University. *11* (4), 67–81.

Wang, S.-Y., S.-T. Chang, C.-L. Wu, Y.-C. Su, S.-L. Huang and Y.H. Kuo. 1998. Three chemical constituents contributing to the color of Taiwania (*Taiwania cryptomerioides* Hayata) heartwood: Hinokinin, Helioxanthin, and Pluviatolide. Q. J. Chin. For. *31* (3), 187–193.

Wenkert, E. and L. Buckwalter. 1972. Carboon-13 nuclear magnetic resonance spectroscopy of naturally occurring substances. X. Pimaradienes. J. Amer. Chem. Soc. *94*, 4367–4368.

Received March 26th 1999

Prof. S.-T. Chang
Dr. S.-Y. Wang
C.-L. Wu
P.-F. Chen
Dept. of Forestry
National Taiwan University
No. 1, Section 4,
Roosevelt Road
Taipei, Taiwan

Prof. Y.-H. Kuo
Dept. of Chemistry
National Taiwan University
No. 1, Section 4
Roosevelt Road
Taipei, Taiwan