

# Chemical Constituents and Mechanisms of Discoloration of Taiwania (*Taiwania cryptomerioides* Hayata) Heartwood

## 1. The Structure Reconfirmation and Conversion Mechanism of Taiwanin A<sup>1)</sup>

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### Keywords

*Taiwania cryptomerioides*

Color substances

Taiwanin A

Conversion mechanism

Taiwanin C

Taiwanin E

### Summary

One of the color substances, taiwanin A, was isolated from Taiwania (*Taiwania cryptomerioides* Hayata) heartwood. According to the <sup>13</sup>C-NMR, <sup>1</sup>H-NMR, HSQC, HMBC, and NOE difference spectroscopy analyses, the diene structure of taiwanin A was reconfirmed to be the *trans-trans* formulation. In addition, it was proven that deep orange crystalline taiwanin A changed to white and pale yellow compounds, taiwanin C and taiwanin E, after light irradiation. The conversion mechanism of taiwanin A into taiwanin C and taiwanin E is also proposed and discussed in this study.

### Introduction

Color is one of the most important properties of wood, and people also use this distinct property as the essential factor to evaluate the value of wood species. Taiwania (*Taiwania cryptomerioides* Hayata) is an economically important tree species indigenous to Taiwan. The heartwood of Taiwania is yellowish red with distinguished purplish pink streaks. According to the custom of Taiwanese, Taiwania should be a fascinating wood species. Unfortunately, the color of Taiwania heartwood is susceptible to change to dull black after exposure to natural environment. This discoloration is a serious problem that decreases the value of Taiwania products. In our previous study (Wang *et al.* 1994), it was proven that the color change in Taiwania heartwood from yellowish red toward bluish green was caused by the combined effects of light and oxygen. Moreover the color was also darkened and eventually turns to black in the presence of moisture. Overall the pronounced discoloration on the surface of Taiwania heartwood was mainly induced by the light with wavelengths in the range of 350–450 nm. On the other hand, we had also effectively retarded the color change of Taiwania by surface treatments (Chang *et al.* 1998). In order to further prevent the discoloration of heartwood, it is expected to identify the extractives contributing to the discoloration and to understand their conversion mechanisms. With regard to the investigation of Taiwania extractives which have been reported by many researches, nine lignans, eight flavones, twenty one sesquiterpenoids, eighteen diterpenoids, three lipids, two cyc-

litols, and one steroid were isolated from Taiwania (Wang *et al.* 1997). However, there are very few researchers, who investigated the relationship between the color variation and extractives of Taiwania. Chang *et al.* (1996) had discriminated preliminarily which fraction of heartwood extractives causes the discoloration. In addition to that study, we try to illustrate the chemical constituents that contribute to the color of Taiwania heartwood and their mechanisms of structure conversion.

### Material and Methods

#### General

HPLC was performed with a Jasco model PU980 pump equipped with a Jasco UV970 UV detector and the column used was a Hibar Lichrosorb Si 60 (25 × 1 cm i.d.). The IR spectrum was recorded on a Bio-rad model FTS-40 spectrophotometer. The EIMS and HREIMS were obtained on Finnigan MAT-958 Mass spectrometer. The NMR spectra were recorded on a Bruker Avance-500 MHz FT-NMR. Light irradiation was made by a plant lamp.

#### Extraction and isolation

Taiwania used in this study was collected from the Experimental Forest of National Taiwan University. Taiwania heartwood chips were prepared from a green cut tree. The air dried chips (5.7 kg) were exhaustively extracted with methanol (MeOH). The extractives were condensed to ca. 286.4 g, then extracted with *n*-hexane (*n*-C<sub>6</sub>H<sub>14</sub>), chloroform (Chl.), ethyl acetate (EtOAc), and methanol (MeOH), successively. After removing solvents from the combined extractives, the *n*-C<sub>6</sub>H<sub>14</sub>, Chl., EtOAc, and MeOH soluble fractions and MeOH insoluble fraction were obtained. Compound **1**, orange crystals (230 mg), was separated during condensing the *n*-C<sub>6</sub>H<sub>14</sub> fraction volume to 50%. White solids and pale yellow solids, compound **2** and compound **3**, were separated from the conversion compounds that derived from compound **1** after irradiating by plant lamp and then purified by using HPLC (CH<sub>2</sub>Cl<sub>2</sub> : EtOAc = 50 : 50, flow rate = 4 ml/min).

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*Light irradiation of compound 1*

Two specimens of compound **1** (35 mg) were dissolved with 50 ml chloroform and 50 ml acetone, respectively. Both solutions were irradiated by plant lamp under stirring. The conversion compounds that induced by light were traced and collected by HPLC.

*Taiwanin A*

Orange crystal, m.p. 201–202 °C; HREIMS for  $C_{20}H_{14}O_6$  found 350.0783, required 350.0786;  $UV\lambda^{CHCl_3}$  max. 268 and 365 nm; IR  $\nu$  max.: 1753, 1630, 1615, 1578, 1485, 1367, 1244, 1165, 1030, 925, 805  $cm^{-1}$ ; NMR: see Table 1.

*Taiwanin C*

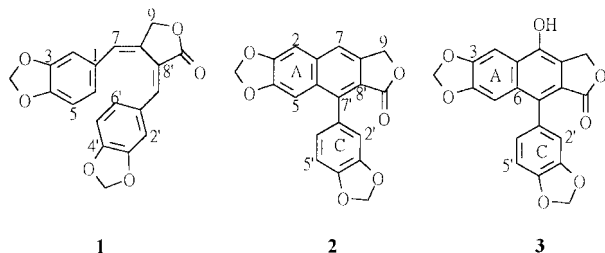
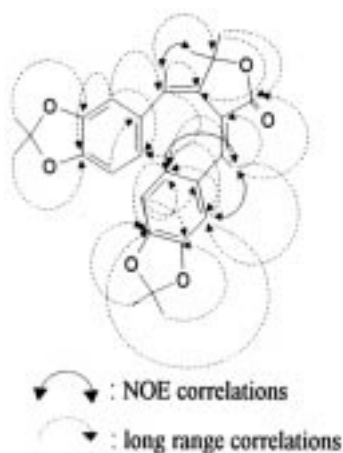
White solids, m.p. 267–270 °C HREIMS for  $C_{20}H_{12}O_6$  found 348.0636, required 348.0630;  $\delta^1H-NMR$  (in  $CDCl_3$ )  $\delta$  (ppm): 5.35 (2H, s, lactone  $CH_2$ ), 6.03 (2H, s, C ring  $O-CH_2-O$ ), 6.06 (2H, d,  $J = 1.3$  Hz, A ring  $O-CH_2-O$ ), 6.77 (1H, d,  $J = 7.8$  Hz, H-6'), 6.78 (1H, s, H-2'), 6.93 (1H, d,  $J = 7.8$  Hz, H-5'), 7.09 (1H, s, H-5), 7.17 (1H, s, H-2) 7.66 (1H, s, H-7).

*Taiwanin E*

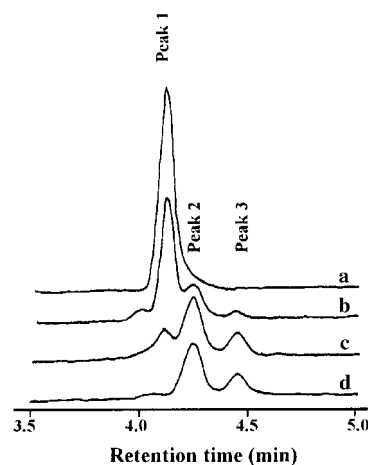
Pale yellow solids, m.p. 267–269 °C HREIMS for  $C_{20}H_{12}O_7$  found 364.3090, required 364.0679;  $\delta^1H-NMR$  (in  $CDCl_3$ )  $\delta$  (ppm): 5.34 (2H, s, lactone  $CH_2$ ), 6.11 (2H, d,  $J = 2.0$  Hz, C ring  $O-CH_2-O$ ), 6.16 (2H, s, A ring  $O-CH_2-O$ ), 6.70 (1H, dd,  $J = 8.0, 2.0$  Hz, H-6'), 6.81 (1H, d,  $J = 2.0$  Hz, H-2'), 6.84 (1H, s, H-5'), 7.02 (1H, s, H-5), 7.60 (1H, s, H-2).

**Results and Discussion**

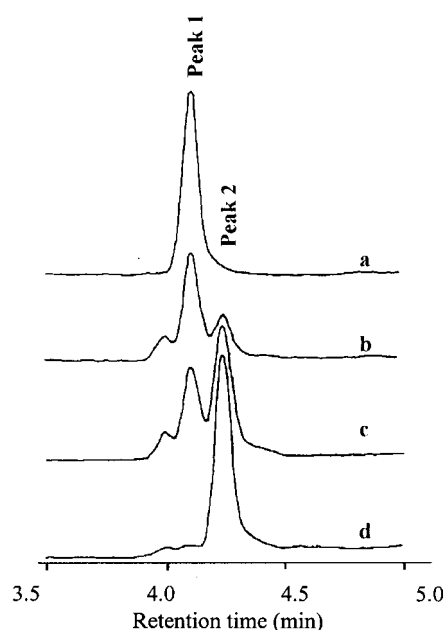
Compound **1** was separated as orange crystals, and its molecular formula,  $C_{20}H_{14}O_6$ , was determined by analyzing its HREIMS molecular ion at  $m/z$  350.0786. The

**Scheme**

**Fig. 1.**  $^1H-^{13}C$  long range correlations and NOE correlations observed for taiwanin A



**Fig. 2.** HPLC chromatograms of taiwanin A in chloroform after plant lamp irradiation for 0 to 120h a: 0h; b: 24h; c: 48h; d: 120h.



**Fig. 3.** HPLC chromatograms of taiwanin A in acetone after plant lamp irradiation for 0 to 120h a: 0h; b: 24h; c: 48h; d: 120h.

$^{13}C-NMR$  spectrum of compound **1** showed 20 carbon signals, including twelve aromatic carbons ( $\delta_C$  149.48, 147.63, 146.37, 146.24, 130.91, 129.49, 125.88, 122.66, 108.83, 107.88, 106.95, 106.95), 4 olefinic carbons ( $\delta_C$  136.60, 128.22, 125.36, 119.48), one carbonyl carbon ( $\delta_C$  173.03), and three methylene carbons ( $\delta_C$  101.29, 100.97, 71.16). In the  $^1H-NMR$  spectrum, the presence of two methylene protons ( $\delta_H$  4.98, 4.98), six aromatic protons ( $\delta_H$  6.68, 6.50, 6.43, 6.43, 6.23, 6.10), four methylenedioxy protons ( $\delta_H$  5.81, 5.81, 5.75, 5.75), and two vinyl protons ( $\delta_H$  7.56, 6.51). The carbon and proton numbers based on the  $^{13}C$ - and  $^1H-NMR$  spectroscopies were consistent with those of HREIMS. The assignment of proton and carbon atoms, moreover, was reconfirmed by two-dimensional homonuclear chemical shift-correlation

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shift data of taiwanin A (ppm, in  $\text{CDCl}_3$ )

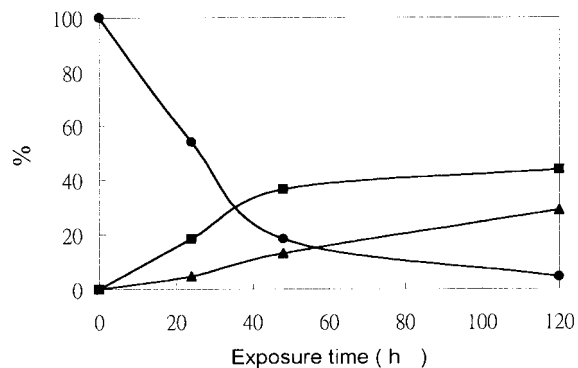
Positions	$^{13}\text{C}$	$^1\text{H}$	NOE
1	130.91		
2	107.88	6.10(s)	
3	147.63		
4	146.37		
5	106.95	6.43(s)	
6	122.66	6.43(s)	
7	125.36	6.51(s)	
8	119.48		
9	71.16	4.98 (d, $J = 4.7$ )	enhance 7-H (10.5 %)
3-O-CH <sub>2</sub> -O-4	100.97	5.75(s)	
1'	129.49		
2'	108.83	6.23 (d, $J = 1.6$ )	
3'	146.24		
4'	149.48		
5'	106.95	6.50 (dd, $J = 8.1\text{ Hz}$ , $J = 1.6\text{ Hz}$ )	
6'	125.88	6.68 (d, $J = 8.1\text{ Hz}$ )	
7'	136.60	7.56(s)	enhance 2'-H (3.9 %) 6'-H (7.1 %)
8'	128.22		
9'	173.03		
3'-O-CH <sub>2</sub> -O-4'	101.29	5.81(s)	

s: singlet, d: doublet, dd: doublet of doublets.

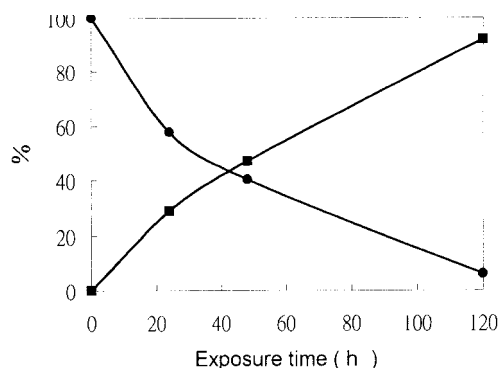
spectroscopies ( $^1\text{H}$ - $^1\text{H}$  COSYs) and heteronuclear single quantum coherence (HSQC). All NMR peaks could be assigned completely as shown in Table 1. The spectral data of compound **1** were in good agreement with taiwanin A (Lin and Wang 1965). However, the determination of the structure of taiwanin A has been confusing. According to the structure assignment of taiwanin A by Lin and Wang (1965), they assigned it to be an  $\alpha$ ,  $\beta$ -bis(piperonylidene)- $\gamma$ -butyrolactone, and the configuration of diene is *cis-trans* form. But, Swoboda *et al.* (1967) proposed that the geometry of the diene should be *cis-cis* formulation. Based on the opinion of Swoboda *et al.* (1967), moreover, they declared *trans-trans* form will not exist because of severe steric interaction between the two aryl groups. In order to distinguish the stereochemistry of taiwanin A, Sai *et al.* (1995) synthesized all isomers of taiwanin A. The chemical shifts of the two vinyl protons of all the isomers prepared were compared with the natural taiwanin A's  $^1\text{H}$ -NMR chemical shifts that reported by Lin and Wang (1965). From x-ray analysis, they assigned taiwanin A was *trans-trans* conformation. For elucidating the detail stereochemistry of taiwanin A, the nuclear Overhauser enhancement (NOE) difference spectroscopy was performed to show the spatial relationships of protons. The results obtained were summarized in Table 1. It was no doubt that taiwanin A should not be

*cis-cis* formulation reported by Swoboda *et al.* (1967) because there were no NOE effects between 7-H and 7'-H. But according to our spectral data obtained from natural taiwanin A, both *trans-trans* and *cis-trans* formulations were possible. Based on the results of Sai *et al.* (1995), we believed that the structure of taiwanin A may be the *trans-trans* conformation. The correlation observed in the heteronuclear multiple bond correlations (HMBC) and the NOE spectrum of taiwanin A was shown in Figure 1.

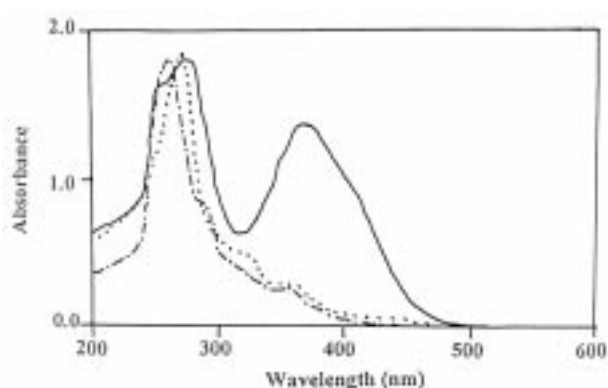
In order to elucidate the photodegradation mechanisms of taiwanin A, we dissolved taiwanin A into two solvents, chloroform and acetone. After irradiation of taiwanin A solutions with plant lamp under stirring, the changes of taiwanin A's structure were monitored by HPLC. Figure 2 demonstrates the HPLC chromatograms of taiwanin A in chloroform after plant lamp irradiation for 0 to 120h (mobile phase:  $\text{CH}_2\text{Cl}_2$ : EtOAc = 50 : 50, flow rate: 4ml/min, injection sample volume: 20 $\mu\text{l}$ ). Before light exposure, there was only one peak 1 (taiwanin A, retention time: 4.1 min) which appeared (a in Fig. 2). However, two new peaks, peak 2 (retention time: 4.3min) and peak 3 (retention time: 4.5min), generated after 24h plant lamp irradiation (b in Fig. 2). Finally, the peak 1 almost disappeared after 120h light irradiation. Compounds **2** and **3** respectively, were collected from peaks 2 and 3 by HPLC. According to the results obtained from  $^1\text{H}$ -NMR, mass spectrum, and the comparison of HPLC retention time using the standard compounds, compounds **2** and **3** were confirmed to be taiwanin C and taiwanin E, whose structures were elucidated by Lin *et al.* (1967). At the same irradiation condition, only the peak 2, taiwanin C, was generated after 120h light exposure in acetone solution (Fig. 3). Moreover, based upon the integral ratios of these three peak areas in different irradiation time, we found out that, after 120h irradiation in chloroform solution (Fig. 4), the conversion ratios of taiwanin A into taiwanin C and taiwanin E were 43.7% and 28.8%, respectively. On the other hand, 92% taiwanin A was converted into taiwanin C after 120h irradiation in acetone (Fig. 5). It was a very interesting phenomenon that, after light irradiation, different conversion products were derived from taiwanin A in these two solvents. In conclusion, it is obvious that after exposure to light deep orange crystalline taiwanin A changed to white



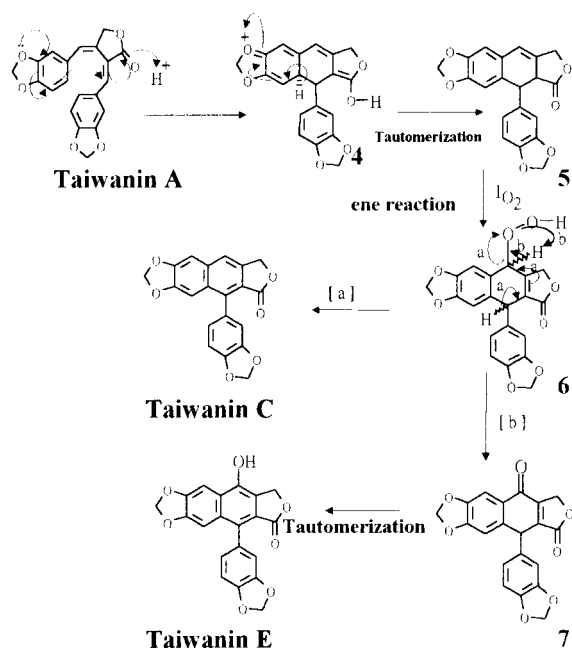
**Fig. 4.** The process of conversion of taiwanin A to taiwanin C and taiwanin E in chloroform during 120h by using plant lamps irradiation (●: taiwanin A; ■: taiwanin C; ▲: taiwanin E).



**Fig. 5.** The process of conversion of taiwanin A to taiwanin C in acetone during 120h by using plant lamps irradiation (●: taiwanin A; ■: taiwanin C).



**Fig. 6.** UV absorbance spectrum (in  $\text{CHCl}_3$ ) of taiwanins A, C, and E (—: taiwanin A; - - -: taiwanin C; · · ·: taiwanin E).



**Fig. 7.** Conversion mechanism of taiwanin A into taiwanin C and taiwanin E.

and pale yellow compounds, taiwanin C and taiwanin E. Furthermore, the UV absorbance spectra of the taiwanins A, C, and E (Fig. 6) also revealed that taiwanin A lost its yellow appearance after conversion into taiwanin C and taiwanin E. Based upon these results, it is speculative that the taiwanins C and E identified in the previous papers are probably derivatives generated during the isolation of Taiwania extractives, which means that they may not be existent in the heartwood of Taiwania.

Figure 7 represents the proposed conversion mechanism of taiwanin A into taiwanin C and taiwanin E. In order to release the strain, taiwanin A underwent Michael addition to give cyclic intermediate 4 which followed tautomerization and aromatization to form intermediate 5. This intermediate revealed a peak with a retention time at 4.0 min in Figures 2 and 3. Due to sensitivity to oxygen, this intermediate has been reported with taiwanin A as sensitizer, the intermediate 5 reacted with  $^1\text{O}_2$  to produce unstable hydrogen peroxide product 6 which followed two pathways. The formation of taiwanin C was *via* 1,4-elimination to remove  $\text{H}_2\text{O}_2$  (path a). *Via* second pathway b, the water elimination led to yield keto intermediate 7 that followed to produce taiwanin E by tautomerization. Because taiwanin C is more stable than intermediate 7, it is only a product in acetone solution or a major product in chloroform solution.

## Conclusion

The heartwood of Taiwania (*Taiwania cryptomerioides* Hayata) is yellowish red with distinguished purplish pink streaks. Unfortunately, the color of Taiwania heartwood is susceptible to change to dull black after exposure it to natural environment. This discoloration is a serious problem that decreases the value of Taiwania products. In order to prevent the discoloration of heartwood, it is a need, to identify the extractives contributing to the discoloration and to understand their conversion mechanisms. One of the color substances, taiwanin A, was isolated from Taiwania heartwood. Although the structure of taiwanin A had been determined already several years ago, the diene structure of Taiwanin A has been confused. According to the  $^{13}\text{C}$ -NMR,  $^1\text{H}$ -NMR, HSQC, HMBC, NOE difference spectroscopy analyses and x-ray diffraction, the diene structure of taiwanin A was reconfirmed to be the *trans-trans* formulation. According to the results of light irradiation experiments, whether in chloroform or acetone, deep orange crystalline taiwanin A could be converted into white taiwanin C and pale yellow taiwanin E. Based on the results obtained it is obvious that the discoloration, generating from the photolysis of Taiwanin A, could be one of the reasons to cause the disappearance of yellowish red color of Taiwania heartwood. Some other reasons leading to the dull black of Taiwania heartwood after exposure to the ambient conditions are under investigation and will be reported in the near future.

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