Antimicrobial compounds as side products from the agricultural processing industry

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Chapter 4

Isolation and elucidation of quinones in Tectona grandis

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Abstract

The compounds deoxylapachol, tectoquinone, 2-hydroxymethylanthraquinone, 3'-OH-deoxyisolapachol (2-[(1*E*)-3-hydroxy-3-methylbut-1-enyl]naphthoquinone), hemitectol (2,2-dimethyl-2*H*-benzo[*h*]chromen-6-ol) and tectol were isolated from *Tectona grandis* sawdust $CHCl_3$-MeOH (1:1) extract. Centrifugal partition chromatography was used to separate these compounds using *n*-hexane-MeOH-H_2O (50:47.5:2.5) as a solvent system. All compounds except tectol showed antifungal activity in a biogram assay against *Aspergillus niger*.

4.1 Introduction

Teak (*Tectona grandis*) wood is commonly used for house construction and furniture in the Indochina region, because this strong hardwood has a beautiful surface and is resistant to mite and fungal damage. For possible new applications, we studied sawdust for the occurrence of interesting phytochemicals. Teak wood contains naphthoquinones (lapachol, deoxylapachol, 5-hydroxylapachol), naphthoquinone derivatives (*α*-dehydrolapachone, *β*-dehydrolapachone, tectol, dehydrotectol), anthraquinones (tectoquinone, 1-hydroxy-2-methylanthraquinone, 2-methyl quinizarin, pachybasin) and also obtusifolin, betulinic acid, trichione, *β*-sitosterol and squalene [Thomson, 1957; Hegnauer, 1973; Singh et al., 1989; Khan and Mlungwana, 1999]. Naphthoquinones have been reported to have antimicrobial activity [Guiraud, et al., 1994; Gafner, et al., 1996]. Lapachol has antitumour activity [Rao, et al., 1968; Rao and Kingston, 1982]. In Chapter 3, it was shown that *T. grandis* sawdust $CHCl_3$-MeOH (1:1) extract has antifungal activity. Here the structures of the active compounds found in this material are isolated and elucidated.

4.2 Materials and Methods

Centrifugal partition chromatography (CPC type LLN, Sanki engineering limited Kyoto, Japan) was used to separate the *T. grandis* chloroform-methanol ($CHCl_3$-MeOH, 1:1) extract using *n*-hexane-methanol-water (*n*-hexane-MeOH-H_2O, 50:47.5:2.5) as a solvent system with *n*-hexane as the mobile phase and MeOH-H_2O as the stationary phase. Ascending mode was used with 2.5 mL/min pump flow rate and 800 rpm rotation speed. After 80 fractions were collected (3 mL per fraction) the system was changed to the descending mode and collecting of fractions continued (fractions 81-90). All fractions were spotted on TLC plates (aluminum sheets silica gel 60 F_{254}, Merck, Darmstadt, Germany). The TLC plates were developed with $CHCl_3$-MeOH (19:1) or petroleum ether-acetone-acetic acid (75:25:1.5) and observed under UV 254 and 366 nm. The TLC plates were sprayed with Anisaldehyde-sulphuric acid reagent or 5% methanolic potassium hydroxide (KOH) [Sherma and Fried, 1991]. The biogram assay was used to test the activity of fractions using *Aspergillus niger* spore suspensions. The active compounds were measured by ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC and HMBC experiments with 300.13 MHz, (Bruker DPX-300), 399.68 MHz, (Bruker DPX-400) and 600.13 MHz (Bruker DPX-600) spectrometer. LC/MS (Agilent, USA) was used to determine the molecular weight of the active compounds. A C_{18} column (length 70 mm, 5 µM particle diameter, Macherey-Nagel, Germany) was used for the analysis with a gradient elution system of water containing 0.1% formic acid

(v/v) (solvent A), and methanol or ethanol containing 0.1% formic acid (v/v) (solvent B). The gradient range was 50-100% of solvent B in 11 min using a flow rate of 0.5 mL/min with an injection volume of 5 µL. All mass spectrometric analyses were performed in a positive atmospheric pressure chemical ionization (APCI) mode.

4.3 Results and discussion

After CPC separation of *T. grandis* extract using *n*-hexane-MeOH-H_2O as the solvent system, it was found that fraction 19-21 (compound I), 25-29 (compound II), 82 (compound III), 84 (compound IV) and 87 (containing both compound V and VI) inhibited *A. niger* growth in the biogram assay. Inhibition was not found with compound VI, which was present in small amounts in fraction 87.

Fraction 19-21 showed an orange colour under UV 366 nm at R_f 0.72 in $CDCl_3$ –MeOH (19:1) and R_f 0.44 in petroleum ether-acetone-acetic acid (75:25:1.5). It also showed a violet colour after spraying with 5% methanolic KOH. The UV spectrum showed absorption at λ_{max} (MeOH + 0.1% formic acid): 205, 250 and 335 nm. The ^{1}H NMR ($CDCl_3$, 300 MHz) showed signals at: δ=1.60 (3H, s, H-5'), 1.71 (3H, s, H-4'), 3.21 (2H, d, *J*=7.3 Hz, H-1'), 5.16 (1H, tm, 7.3 Hz, 1.4 Hz, H-2'), 6.70 (1H, t, *J*=1.7 Hz, H-3), 7.66 (2H, m, H-6, H-7), 7.99 and 8.03 (2H, m, H-5, H-8). These data and data from LC/MS: 227 $[M+H]^+$, correspond with deoxylapachol (Figure 4.1).

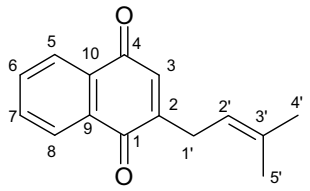

Figure 4.1 The structure of deoxylapachol (compound I)

Fraction 25-29 showed a red-orange colour under UV 366 nm at R_f 0.72 in $CDCl_3$ – MeOH (19:1) and R_f 0.37 in petroleum ether-acetone-acetic acid (75:25:1.5). The UV spectrum showed absorption at λ_{max} (MeOH + 0.1% formic acid): 205, 255 and 330 nm. The ^{1}H NMR ($CDCl_3$, 300 MHz) showed signals at: δ=2.47 (3H, s, H-1'), 7.53 (1H, d, *J*=7.9 Hz, H-3), 7.72

(2H, m, H-6, H-7), 8.04 (1H, s, H-1), 8.14 (1H, d, *J*=7.9 Hz, H-4), 8.23 (2H, m, H-8, H-5). These data and the result from LC/MS: 223 $[M+H]^+$, correspond with tectoquinone (Figure 4.2).

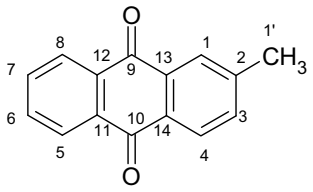

Figure 4.2 The structure of tectoquinone (compound II)

Fraction 82 contained active compound III at R_f = 0.29 which presented a bright yellow colour on TLC silica gel plate when developed with $CDCl_3$ –MeOH (19:1) and showed a bright orange colour under UV 366 nm, a purple colour after spraying with AS, and a red colour after spraying with 5% methanolic KOH. The UV spectrum showed absorption at λ_{max} (EtOH + 0.1% formic acid): 205, 257 and 330 nm. ^{1}H NMR and ^{13}C NMR data and ^{1}H-^{1}H COSY, HSQC and HMBC experiments ($CDCl_3$, 600 MHz) were measured with 600 MHz Bruker DPX-600 spectrometer (Tables 4.1 and 4.2). These data and the data from LC/MS: 240 $[M+H]^+$, correspond with 2-hydroxymethylanthraquinone is proposed (Figure 4.3).

Table 4.1 COSY ($CDCl_3$, 600 MHz) correlations of 2-hydroxymethyl anthraquinone (compound III)

H		3	6 and 7	1'
	δ_H	7.82 (1H, m)	7.81 (2H, m)	4.90 (2H, s)
1	8.29 (1H, s)			*
3	7.82 (1H, m)			*
6 and 7	7.81 (2H, m)			
4, 5 and 8	8.32 (3H, m)	*	*	
1'	4.90 (2H, s)			

Table 4.2 HSQC (*) and HMBC (Δ) ($CDCl_3$, 600 MHz) correlations of 2-hydroxymethylanthraquinone (compound III)

C/H		1	3	6 and 7	4, 5 and 8	1'
	δ_C/δ_H	8.29 (1H)	7.82 (1H, m)	7.81 (2H, m)	8.32 (3H, m)	4.90 (2H, s)
1	124.91	*	Δ			Δ
2	147.58					Δ
3	131.97	Δ	*			Δ
4	127.73				*	
5	127.27			Δ	*	
6	134.19			*	Δ	
7	134.11			*	Δ	
8	127.25			Δ	*	
9	183.17	Δ			Δ	
10	182.93				Δ	
11 and 12	133.53 and 133.54			Δ	Δ	
13	133.64				Δ	
14	132.72	Δ	Δ			
1'	64.40					*

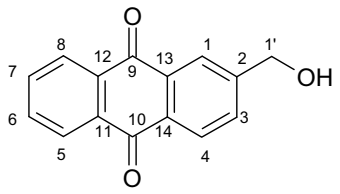

Figure 4.3 The structure of 2-hydroxymethylanthraquinone (compound III)

Fraction 84 contained active compound IV at R_f 0.31 which showed a bright yellow colour on the TLC silica gel plate when developed with $CDCl_3$ –MeOH (19:1), a dark orange colour under UV 366 nm and a dark red colour after spraying with 5% methanolic KOH. The UV spectrum showed absorption at λ_{max} (EtOH + 0.1% formic acid): 208, 252 and 300, 345 nm. ^1H NMR and ^{13}C NMR data and ^1H-^1H COSY, HSQC and HMBC experiments ($CDCl_3$, 600 MHz) were measured with 600 MHz Bruker DPX-600 spectrometer (Tables 4.3 and 4.4). LC/MS showed 225 $[M+H-18]^+$. Based on the spectral data, the novel structure IV (Figure 4.4)

is proposed (2-[(1*E*)-3-hydroxy-3-methylbut-1-enyl] naphthoquinone), which we gave the trivial name 3'-OH-deoxyisolapachol.

Table 4.3 COSY ($CDCl_3$, 600 MHz) correlations of 3'-OH-deoxyisolapachol (compound IV)

H		6 and 7	1'
	δ_H	7.75 (2H, m)	6.81 (1H, d, *J*=16.3 Hz)
3	6.98 (1H, s)		
5	8.07 (1H, m)	*	
6 and 7	7.75 (2H, m)		
8	8.12 (1H,m)	*	
1'	6.80 (1H, d, *J*=16.3 Hz)		
2'	6.83 (1H, d, *J*=16.3 Hz)		*
4' and 5'	1.44 (6H, s)		

Table 4.4 HSQC (*) and HMBC (Δ) ($CDCl_3$, 600 MHz) correlations of 3'-OH-deoxyisolapachol (compound IV)

C/H		3	5	6 and 7	8	1'	2'	4' and 5'
	δ_C/δ_H	6.98 (1H, s)	8.07 (1H, m)	7.75 (2H, m)	8.12 (1H,m)	6.80 (1H, d, *J*=16.3 Hz)	6.83 (1H, d, *J*=16.3 Hz)	1.44 (6H, s)
1	185.40					Δ		
2	143.81	Δ					Δ	
3	131.28	*						
4	184.90	Δ						
5	125.93		*	Δ				
6	133.90			*	Δ			
7	133.77		Δ	*				
8	126.77				*			
9	132.30		Δ	Δ				
10	132.40	Δ		Δ	Δ			
1'	118.52	Δ				*		
2'	147.44						*	Δ
3'	71.61							Δ
4' and 5'	29.55							*,Δ

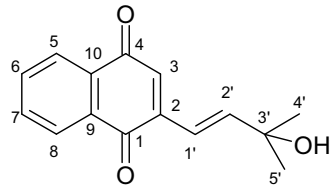

Figure 4.4 The structure of 3'-OH-deoxyisolapachol (compound IV)

Fraction 87 showed activity and contained two compounds (compound V and VI). Compound V at R_f 0.47 showed a violet colour under UV 366 nm, a blue-gray colour after spraying with AS and a red-purple colour after spraying with 5% methanolic KOH. The UV spectrum showed absorption at λ_{max} (EtOH + 0.1% formic acid): 222, 273 and 360 nm. ^{1}H NMR and ^{13}C NMR data and ^{1}H-^{1}H COSY, HSQC and HMBC experiments (MeOD, 400 MHz) were measured with 400 MHz Bruker DPX-400 spectrometer (Tables 4.5 and 4.6). LC/MS showed 227 $[M+H]^{+}$. Based on the spectral data, the structure of the novel compound (Figure 4.5, compound V) is proposed (2,2-dimethyl-2*H*-benzo[*h*]chromen-6-ol). This compound was given the trivial name hemitectol.

Table 4.5 COSY (MeOD, 400 MHz) correlations of hemitectol (compound V)

H		6 and 7	2'
	δ_H	7.39 (2H, m)	5.68 (1H, d, *J*=9.6 Hz)
3	6.53 (1H, s)		
5 and 8	8.04 (2H, m)	*	
6 and 7	7.39 (2H, m)		
1'	6.36 (1H, d, *J*=9.6 Hz)		*
2'	5.68 (1H, d, *J*=9.6 Hz)		
4' and 5'	1.45 (6H, s)		

Table 4.6 HSQC (*) and HMBC (Δ) (MeOD, 400 MHz) correlations of hemitectol (compound V)

C/H		3	5 and 8	6 and 7	1'	2'	4' and 5'
	δ_C/δ_H	6.53 (1H, s)	8.04 (2H, m)	7.39 (2H, m)	6.36 (1H, d, *J*=9.7 Hz)	5.68 (1H, d, *J*=9.7 Hz)	1.45 (6H, s)
1	142.13	Δ			Δ		
2	116.74				Δ	Δ	
3	106.99	*			Δ		
4	147.80	Δ					
5	123.13		*	Δ			
6	126.49		Δ	*			
7	125.81		Δ	*			
8	122.45		*	Δ			
9 and 10	126.95 and 127.14						
1'	123.97	Δ			*		
2'	131.27					*	Δ
3'	77.07				Δ	Δ	Δ
4' and 5'	27.69 and 27.84					Δ	*, Δ

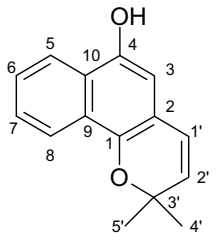

Figure 4.5 The structure of hemitectol (compound V)

Compound VI at R_f = 0.77 showed a violet colour under UV 366 nm, a gray colour after spraying with AS, and a violet colour after spraying with 5% methanolic KOH. The UV spectrum showed absorption at λ_{max} (EtOH + 0.1% formic acid): 230, 275 and 362 nm. ^{1}H NMR and ^{13}C NMR data and ^{1}H-^{1}H COSY, HSQC and HMBC experiments ($CDCl_3$, 600 MHz) were measured with 600 MHz Bruker DPX-600 spectrometer (Tables 4.7 and 4.8). LC/MS showed 451 $[M+H]^+$. The spectral data correspond with tectol (Figure 4.6).

Table 4.7 COSY ($CDCl_3$, 600 MHz) correlations of tectol (compound VI)

H		6 and 7	2'	5'
	δ_H	7.46 (2H, m)	5.56 (1H, d, *J*=9.8 Hz)	1.46 (3H, s)
5	8.15 (1H, d, *J*= 7.9 Hz)	*		
8	8.18 (1H, d, *J*=7.7 Hz)	*		
6 and 7	7.46 (2H, m)			
1'	5.86 (1H, d, *J*=9.8 Hz)		*	
2'	5.56 (1H, d, *J*=9.8 Hz)			
4'	1.50 (3H, s)			*
5'	1.46 (3H, s)			

Table 4.8 HSQC (*) and HMBC (Δ) ($CDCl_3$, 600 MHz) correlations of tectol (compound VI)

C/H		5	8	6 and 7	1'	2'	4'	5'
	δ_C/δ_H	8.15 (1H, d, *J*= 7.9 Hz)	8.18 (1H, d, *J*=7.7 Hz)	7.46 (2H, m)	5.86 (1H, d, *J*=9.8 Hz)	5.56 (1H, d, *J*=9.8 Hz)	1.50 (3H, s)	1.46 (3H, s)
1	143.20		Δ		Δ			
2	117.40					Δ		
3	114.00				Δ			
4	145.30	Δ						
5	123.57	*		Δ				
6	126.46		Δ	*				
7	126.78	Δ		*				
8	122.68		*	Δ				
9 and 10	127.17 and 127.27			Δ				
1'	122.37				*			
2'	130.92					*	Δ	Δ
3'	76.73				Δ	Δ	Δ	Δ
4'	27.84					Δ	*	Δ
5'	27.67					Δ	Δ	*

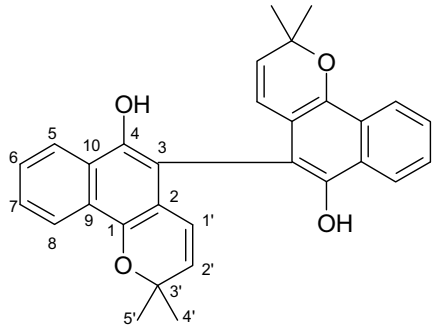

Figure 4.6 The structure of tectol (compound VI)

4.4 Conclusion

Centrifugal partition chromatography (CPC) was used successfully to separate the active compounds from *T. grandis* extract. The biogram assay was used for fast screening of the antifungal compounds from CPC fractions. Six compounds were found and were identified as deoxylapachol, tectoquinone, tectol, hemitectol, 2-hydroxymethylanthraquinone and 3'-OH-deoxyisolapachol, all of which except tectol showed zones of inhibition in the biogram assay. Some of these compounds were tested further for antimicrobial activities (Chapters 5 and 7).