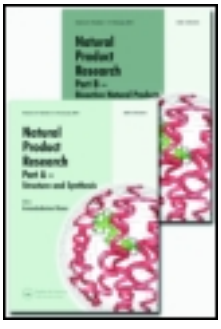


Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Xanthones from roots of Calophyllum thwaitesii and their bioactivity

H.R.W. Dharmaratne [a], M.T. Napagoda [a] & S.B. Tennakoon [a]
[a] Natural Products Programme, Institute of Fundamental Studies, Kandy 20000, Sri Lanka
Published online: 29 Oct 2009.

To cite this article: H.R.W. Dharmaratne , M.T. Napagoda & S.B. Tennakoon (2009) Xanthones from roots of Calophyllum thwaitesii and their bioactivity, Natural Product Research: Formerly Natural Product Letters, 23:6, 539-545, DOI: 10.1080/14786410600899118

To link to this article: http://dx.doi.org/10.1080/14786410600899118

Natural Product Research
Vol. 23, No. 6, 15 April 2009, 539–545

Taylor & Francis
Taylor & Francis Group

Xanthones from roots of *Calophyllum thwaitesii* and their bioactivity

H.R.W. Dharmaratne*, M.T. Napagoda and S.B. Tennakoon

Natural Products Programme, Institute of Fundamental Studies, Kandy 20000, Sri Lanka

(*Received 26 March 2005; final version received 26 December 2005*)

Extracts from three *Calophyllum* species endemic to Sri Lanka were tested for antifungal and antioxidant activities. Of them, only the root methanol extract of *Calophyllum thwaitesii* showed activity, and the active extract on activity guided fractionation yielded four antifungal active and three inactive xanthones. Antifungal active xanthones were identified as 1,6-dihydroxy-5-methoxyxanthone, 1-hydroxy-5,6-dimethoxyxanthone, 1-hydroxy-5-methoxyxanthone and 1-methoxy-5-hydroxyxanthone, using spectroscopic methods and comparison with literature data. Inactive compounds were identified as 1-hydroxy-7-methoxyxanthone, 1,5-dihydroxy-6-methoxyxanthone and 1,7-dihydroxy xanthone. This is the first report of above xanthones except the latter from *C. thwaitesii*. Further, five of the above xanthones along with thwaitesixanthone and calothwaitesixanthone, which have been previously reported from the root bark of the same species, showed free radical scavenging properties when tested with DPPH. Further, this is the first report of methylated xanthones from *C. thwaitesii*. Previous work on stem bark, root bark and the stem of the same species yielded only nonmethylated xanthones, indicating the absence of methylating enzymes in the plant. However, this new finding suggests the presence of methylating enzymes in the root stem of *C. thwaitesii*.

Keywords: *Calophyllum thwaitesii* Planch and Triana; root bark; xanthones; antifungal activity; antioxidant activity

1. Introduction

Calophyllum species are found to be a rich source of secondary metabolites, such as xanthones, terpenoids, coumarins and chromene acids (Dharmaratne, Tan, Marasinghe, & Pezzuto, 2002). However, limited attention has been given to their biological activity. In the present study we have focused on the antifungal activity of *Calophyllum* species against two fungal strains, and the free radical scavenging properties of the isolates. *Cladosporium* and *Aspergillus* are common fungal genera occurring both indoors and outdoors. *Cladosporium* normally exists as a saprophyte or as a weak plant pathogen causing numerous scab diseases as well as leaf spots and blights (Coomaraswamy, 1979). *Aspergillus* typically occurs in the soil and also causes post-harvest diseases in fruits and

*Corresponding author. Email: hrwd13@hotmail.com

ISSN 1478–6419 print/ISSN 1029–2349 online

DOI: 10.1080/14786410600899118
http://www.informaworld.com

vegetables (Prescott, Harley, & Klein, 1979). Therefore, it is important to search for naturally occurring antifungal compounds with the hope of controlling plant pathogen-based diseases originating from these strains, which cause tremendous loss of post-harvest fruits and vegetables. Further, antioxidant activity of isolates were also investigated.

2. Results and discussion

Extracts of *C. thwaitesii* (root stem), *C. cordato-oblongum* (twigs and stem bark) and *C. moonii* (stem) were tested against *Cladosporium* and *Aspergillus* strains using TLC bioassay and disc diffusion method, respectively. However, only the methanol extract of the root stem of *C. thwaitesii* showed antifungal activity (200 μg/spot) against both of the strains. The active extract was subjected to activity guided fractionation. Column chromatography of the methanol extract of the root stem of *C. thwaitesii* gave three active fractions against *Cladosporium* as well as *Aspergillus* strains. Further, column chromatography of the above active fractions, followed by PTLC and HPLC, gave four active compounds against the fungal strains and three inactive compounds (see Table 1).

^{1}H NMR spectra of above yellow crystalline compounds indicated them to be xanthones, and their CIMS showed M^+ values of *m*/*z* 242, 258 and 272. The most active compound (**1**) had M^+ of *m*/*z* 272. Its ^{13}C NMR spectrum indicated the presence of 15 carbon atoms, including a carbonyl carbon of the xanthone moiety appearing at δc 182.9, 2 aryl methoxy groups at δc 57.5 and 62.1, and 12 aromatic carbon atoms (see Table 2). Further, five of the aromatic carbons were found to be protonated. In the ^{1}H NMR spectrum of **1**, the low field ^{1}H signal at δH 12.98 indicated the presence of a chelated hydroxy group adjacent to the carbonyl group at C-l, and two 3H signals at δH 3.93 and 4.01 for the two aryl OMe groups. Further studies on HMQC and HMBC experimental data indicated the positions of two methoxy groups to be at C-6 and C-5 of xanthone, respectively. Considering the above information, the structure of the antifungal compound **1** was proposed to be 1-hydroxy-5,6-dimethoxyxanthone. The next antifungal active

Table 1. Antifungal and antioxidant activities of *Calophyllum* xanthones.

Compound	Antifungal activity		Antioxidant activity
	Cladosporium (μg/spot)	*Aspergillus* diameter (cm)	
C. thwaitesii (root stem MeOH extract)	200	1.56	Active
1,6-dihydroxy-5-methoxyxanthone	200	1.22	Active
1-hydroxy-5,6-dimethoxyxanthone	50	1.07	Inactive
1-hydroxy-5-methoxyxanthone	200	1.05	Active
1-methoxy-5-hydroxyxanthone	200	1.00	Active
1-hydroxy-7-methoxyxanthone	Inactive	Inactive	Inactive
1,7-dihydroxyxanthone	Inactive	Inactive	Active
I,5-dihydroxy-6-methoxyxanthone	Inactive	Inactive	Active
Thwaitesixanthone	Inactive	Inactive	Active
Calothwaitesixanthone	Inactive	Inactive	Active
Benlate	Active	4.7	–

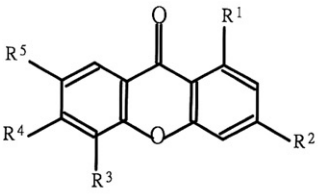
compound, **2**, had an M^+ of *m*/*z* 258. Its ^{13}C NMR spectrum indicated the presence of the carbonyl carbon of the xanthone moiety at δc 180, and an aryl methoxy group at δc 62.8. In the ^1H NMR spectrum of **2**, the low field ^1H signal at δH 12.87 indicated the presence of a chelated hydroxy group adjacent to carbonyl group at C-l, and the 3H signal at δH 4.05 for an aryl OMe group. The similarity between the ^1H and ^{13}C NMR spectra of **1** and **2** suggested **2** to be a monomethyl derivative of **1**. Therefore, **2** was methylated using diazomethane and compared with **1**. TLC comparison showed these compounds to be the same, and confirmed our suggestion. Further studies on HMQC and HMBC experimental data suggested that the second antifungal compound **2** to be 1,6-dihydroxy-5-methoxyxanthone. The next active compound, **3**, had an M^+ of *m*/*z* 242. Its ^{13}C NMR spectrum indicated the presence of 14 carbon atoms, including a signal due to an aryl methoxy group at δc 63.1. However, no peak was observed due to a chelated hydroxy group in the ^1H NMR spectrum of **3**. These observations suggested the presence of a methoxy group at C-l of the xanthone moiety. Further studies on HMQC and HMBC experimental data confirmed the single methoxy group to be at C-l position of the xanthone and the structure of the antifungal compound **3** was proposed as l-methoxy-5-hydroxyxanthone. The next antifungal active compound, **4**, also had an M^+ of *m*/*z* 242. Its ^{13}C NMR spectrum indicated the presence of an aryl methoxy group at δc 60.9. In the ^1H NMR spectrum of **4**, the low field 1H signal at δc 12.81 indicated the presence of a chelated hydroxy group adjacent to carbonyl group at C-l, and the 3H signal at δH 3.95 for an aryl OMe group. The similarity between the ^1H NMR spectra of **3** and **4** suggested them to be different monomethyl derivatives from a single compound. Methylation of **3** and **4** gave the same compound and therefore, the structure of **4** was confirmed as 1-hydroxy-5-methoxyxanthone. Structures of inactive compounds were confirmed as l-hydroxy-7-methoxyxanthone (**5**), 1,7-dihydroxyxanthone (**6**) and 1,5-dihydroxy-6-methoxyxanthone (**7**) using spectroscopic data (see Tables 2 and 3). This is the first report of the isolation of xanthones **1**, **2**, **3**, **4**, **5** and **7** from *C. thwaitesii*. Further, this is the first time where

Table 2. ^{13}C NMR values of xanthones.

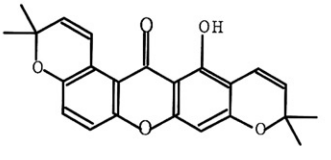
Carbon number	Compound						
	1	**2**	**3**	**4**	**5**	**6**	**7**
1	162.5	161.8	148.2	162.7	162.1	161.0	161.3
2	110.6	110.2	132.9	110.2	110.4	109.3	110.4
3	136.7	136.4	127.9	140.1	136.8	136.4	137.5
4	106.7	106.4	136.4		107.3	106.9	105.8
4a	156.0	155.6	157.3		156.4	156.3	
5	149.5	145.3	169.9		119.6	118.9	
6	149.2		119.3	122.0	125.9	125.1	
7	113.3	114.1	125.9	128.1	156.6	153.8	
8	121.6	123.2	115.7	129.7	104.9		121.4
8a	116.1		123.5	125.8	121.1	120.8	
9	182.9	180.0	179.1		182.3	182.2	
9a	109.4		118.0		108.9	108.1	107.0
10a	151.5		152.5		151.3	150.1	
OMe	62.1 (C-5) 57.5 (C-6)	62.8 (C-5)	63.1 (C-l)	60.9 (C-5)	56.2 (C-7)		57.2 (C-6)

Table 3. ^{1}H NMR data of xanthones in $CDCl_3$ (coupling constants (Hz) are in parentheses).

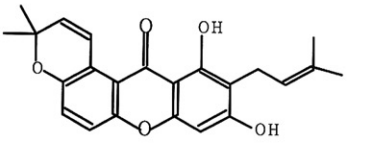
	1	2	3	4	5	6	7
1-OH	12.98 (s)	12.87 (s)	–	–	–	–	–
1-OMe	–	–	3.95	12.81(s)	12.6 (s)	13.0 (s)	11.97 (s)
2-H	6.73 (d, J = 8.25)	6.68 (d, J = 8.2)	7.58 (m)	7.30 (d, J = 9.6)	6.75 (d, d)	6.71 (d, J = 0.71)	6.76 (d, J = 8.7)
3-H	7.52 (t, J = 8.25, 16.55, 8.3)	7.51 (t, J = 8.3, 16.7, 8.3)	8.2 (d, d)	7.74 (t, J = 8.4 15.6,7.2)	7.53 (d, d)	7.51 (m)	7.59 (t, J = 8.7, 17.4, 8.7)
4-H	6.81 (d, J = 8.35)	6.82 (d, J = 8.4)	7.71 (m)	6.91 (d, J = 8.4)	6.85 (d, d)	6.91 (d, J = 0.84)	6.88 (d, d)
5-H	–	–	–	–	7.32 (d, J = 2.7)	7.35 (d, J = 9.0)	
5-OH	–	–	–	–	–	–	11.78 (s)
5-OMe	4.01	4.01	–	3.95	–	–	–
6-H	–	–	7.48 (d, J = 8.41)	7.48 (d, J = 8.4)	7.29 (d, d)	7.27 (m)	–
6-OMe	3.93	–	–	–	–	–	3.94
7-H	7.19 (d, J = 8.9)	7.13 (d, J = 9.1)	7.35 (t, J = 9.0, 16.71,7.64)	7.35 (t, J = 8.4 15.6, 7.2)	–	–	26 (s)
7-OMe	–	–	–	–	3.89	–	–
8-H	7.38 (d, J = 8.9)	7.33 (d, J = 9.l)	7.21 (d, J = 9.15)	8.27 (d, J = 7.2)	7.55 (m)	–	32 (d, J = 8.7)



1. 1-hydroxy-5,6-dimethoxyxanthone (R^1= OH, R^3 = R^4= OMe, R^2 = R^5= H)
2. 1,6-dihydroxy-5-methoxyxanthone (R^1= R^4 = OH, R^3= OMe, R^2 = R^5= H)
3. 1-methoxy-5-hydroxyxanthone (R^1=OMe, R^3 = OH, R^2 = R^4 = R^5 = H)
4. 1-hydroxy-5-methoxyxanthone (R^1= OH, R^3= OMe, R^2 = R^4 = R^5 = H)
5. 1-hydroxy-7-methoxyxanthone (R^1= OH, R^5= OMe, R^2 = R^3 = R^4 = H)
6. 1,7-dihydroxyxanthone (R^1= R^5 = OH, R^2= R^3 = R^4 = H)
7. 1,5-dihydroxy-6-methoxyxanthone (R^1= R^3 = OH, R^4= OMe, R^2 = R^5= H)



8. thwaitesixanthone



9. calothwaitesixanthone

Figure 1. Xanthones isolated from *Calophyllum thwaitesii*.

methylated xanthones have been reported from this species, even though some of the above are reported from other *Calophyllum* species (Figure 1) (Sultanbawa, 1980). In previous investigations, on the basis of the nonappearance of methylated xanthones in *C. thwaitesii*, it was forecasted that methylating enzymes are absent in this species (Dharmaratne, Sotheeswaran, Balasubramaniam, & Reisch, 1986). However, our present finding indicates the presence of methylating enzymes in the root stem of *C. thwaitesii*.

Thwaitesixanthone and calothwaitesixanthone showed no activity against tested fungal strains. In the present study, we have observed that our modified disc diffusion method, which commonly used in bacterial studies (Barnett, 1989) working well with the filamentous fungus *Aspergillus*.

All the above xanthones, along with thwaitesixanthone and calothwaitesixanthone (Dharmaratne, Sotheeswaran, Balasubramaniam, & Reisch, 1986), were subjected to antioxidant activity studies, using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method (Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002). Xanthones were spotted on thin layer chromatography (TLC) plates, and exposed to a free radical reagent DPPH to visualise antioxidant activity (Guo, Lee, Chiang, Lin, & Chang, 2001; Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002). Interestingly all the xanthones with free hydroxy groups, and the positive control tocopherol gave yellow spots, showing neutralisation of the dark purple free radical and indicating their free radical scavenging properties. Our observations suggested that the above active xanthones belong to primary antioxidants which are responsible for the chain breaking and free radical scavenging (Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002).

3. Experimental

3.1. *General procedure*

^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DPX-300 (300 MHz for ^{1}H NMR and 75.45 MHz for ^{13}C NMR) and Bruker Avance DPX-500 (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometers using TMS as internal standard in $CDCl_3$ low resolution MS analysis was performed on a AQA LCMS system (Thermoquest, San Jose, CA, USA) and a Finnigan HPLC system.

3.2. *Plant material*

C. cordato-oblongum, *C. moonii*, and *C. thwaitesii* were identified and collected in 1996 from the Kanneliya forest in the Southern province of Sri Lanka by Mr. Shantha Ekanayake (Institute of Fundamental Studies), and the plant specimens were compared with the herbarium specimens at the Royal Botanic Gardens, Peradeniya, Sri Lanka. Dried plant material were powdered and extracted at room temperature using a linear shaker with hexane, methylene chloride and methanol respectively. Silica gel column chromatography, PTLC and HPLC were used to fractionate and isolate pure compounds. Structure elucidation of isolates were carried out using spectroscopic data and comparison with literature.

3.3. *Antifungal screening*

TLC bioassay (Homans & Fuchs, 1970) was used to check the antifungal activity of extracts and pure compounds against *Cladosporium*. In this method, about 10 mL of Czepak Dox Broth (CDB) was added to previously prepared *Cladosporium* cultures and shaken well till the water turned cloudy. The spores were then filtered into the spraying apparatus through glass wool or muslin cloths. Concentration of the spore suspension was adjusted to 40–50 spores per field at X 400 by adding prepared CDB medium. This spore suspension was sprayed on to previously spotted TLC/PTLC plates which were air-dried for about 6 h. The plates were incubated at room temperature in a moisture chamber for 2 days and thereafter observed for inhibition in the growth.

The disc diffusion method, which is commonly used for bacteria (Banett, 1989) and also for yeast like fungi, was modified in order to use for filamentous fungi like *Aspergillus*. In this method, a liquid culture of *Aspergillus* on CDB was prepared by inoculating 7 day old fungus grown on potato dextrose agar. Fractions/pure compounds (1 mg) each were dissolved in 100 μL of MeOH/EtOAc, and sterile disk papers (diameter of 7 mm) were soaked with 20 μL of the prepared solution and left to dry completely. Meanwhile CDA medium was prepared, autoclaved and cooled to about 45°C and then inoculated with the liquid culture of *Aspergillus* (0.5 mL of liquid culture for 25 mL of CDA medium). Then the medium was poured into sterilised petri dishes (20 mL each) and left until solidified. After solidification, dried disc papers were placed on the inoculated medium, sealed with parafilm and kept for 24 h in the refrigerator at 4°C. Thereafter, the plates were transferred into an incubator (30°C). Readings were taken after 3 and 5 days. Diameter of the inhibition zones were measured along the two diameters at right angle to each other. Two replicates were used for each sample. Benlate was used as the positive control while sterile distilled water was used as the negative control.

Acknowledgements

The authors thank Prof. N. P. D. Nanayakkra and Dr F. Wiggers for providing NMR spectra and Dr M. Ganzera for performing the MS analyses at the National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA.

References

Barnett, M.E. (1989). *Microbiology laboratory exercises*. Iowa, USA: Wm C. Brown.

Coomaraswamy, U. (1979). *A handbook to the fungi parasitic on the plants of Sri Lanka*. Sri Lanka: National Science Council of Sri Lanka.

Dharmaratne, H.R.W., Sotheeswaran, S., Balasubramaniam, S., & Reisch, J. (1986). Xanthones from roots of three *Calophyllum* species. *Phytochemistry, 25*, 1957–1959.

Dharmaratne, H.R.W., Tan, G.T., Marasinghe, G.P.K., & Pezzuto, J.M. (2002). Inhibition of HIV-1 reverse transcriptase and HIV-1 replication by *Calophyllum* coumarins and xanthones. *Planta Medica, 68*, 86–87.

Guo, J.-T., Lee, H.-L., Chiang, S.-H., Lin, F.-1., & Chang, C.-Y. (2001). Antioxidant properties of the extracts from different parts of broccoli in Taiwan. *Journal of Food and Drug Analysis, 9*, 96–101.

Homans, A.L., & Fuchs, A. (1970). Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. *Journal of Chromatography, 51*, 327–329.

Koleva, I.I., van Beek, T.A., Linssen, J.P.H., de Groot, A., & Evstatieva, L.N. (2002). Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis, 13*, 8–17.

Prescott, L.M., Harley, J.P., & Klein, D.A (1979). *Microbiology* (3rd ed.). Iowa, USA: Wm. C. Brown.

Sultanbawa, M.U.S. (1980). Xanthonoids of tropical plants. *Tetrahedron, 36*, 1465–1506.