PERGAMON

Phytochemistry 55 (2000) 833–836

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Xanthones from *Calophyllum teysmannii* var. *inophylloide*☆

Anake Kijjoa [a,*], Maria José Gonzalez [b], Madalena M.M. Pinto [b], Artur M.S. Silva [c], Choojit Anantachoke [d], Werner Herz [e]

[a] *Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, 4099-003 Porto, Portugal*
[b] *Centro de Estudos de Química Orgânica, Fitoquímica e Farmacologia da Universidade do Porto, 4050 Porto, Portugal*
[c] *Departamento de Química, Universidade de Aveiro, 3810 Aveiro, Portugal*
[d] *Wood Products R&D Division, Royal Forest Department, Phahon Yothin Road, Chatuchak, Bangkok 10900, Thailand*
[e] *Department of Chemistry, The Florida State University, Tallahassee, FL 32306-4390, USA*

Received 3 January 2000; received in revised form 11 April 2000

Abstract

Further study of the wood of *Calophyllum teysmannii* Miq. var. *inophylloide* yielded xanthones 7-hydroxy-1,2,8-trimethoxyxanthone, 6-hydroxy-1,2,5-trimethoxyxanthone, and 2-carbomethoxy-6-methoxyxanthone in addition to 3,8-dihydroxy-1,2,4-trimethoxyxanthone, 3-hydroxy-2,4-dimethoxyxanthone, 1,7-dihydroxy-3-methoxyanthone (gentisin) and 2-hydroxyxanthone. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Calophyllum teysmannii* var. *inophylloide*; Guttiferae; Xanthones; 7-Hydroxy-1,2,8-trimethoxyxanthone; 6-Hydroxy-1,2,5-trimethoxyxanthone; 2-Carbomethoxy-6-methoxyxanthone; 3,8-Dihydroxy-1,2,4-trimethoxyxanthone; 3-Hydroxy-2,4-dimethoxyxanthone; 1,7-Dihydroxy-3-methoxyxanthone; 2-Hydroxyxanthone

1. Introduction

Leaves, twigs, latex or stem bark of collections of *Calophyllum teysmannii* Miq. var. *inophylloide* (King) PF Stevens from Malaysia and Indonesia have yielded dipyranocoumarins which act as potent inhibitors of HIV-1 RT (Patil et a1., 1993; Gustafson et al., 1994; Fuller et al., 1994; McKee et al., 1996, 1998; Cao et al., 1996, 1998a,b). However, there were no reports on constituents of the wood.

2. Results and discussion

We recently reported isolation and immunomodulatory activity of nine known xanthones from the wood of a collection of *C. teysmannii* var. *inophylloide* from Narathiwat Province, Southern Thailand (Gonzalez et al., 1999) and subsequently described three new xanthones and several other constituents from the same collection (Kijjoa et al., 2000). Further study of the remaining fractions has now led to the isolation of three more new xanthones, i.e. 7-hydroxy-1,2,8-trimethoxyxanthone (**1**), 6-hydroxy-1,2,5-trimethoxyxanthone (**2**), and 2-carbomethoxy-6-methoxyxanthone (**6a**). Previously known xanthones from this fraction not previously found in *C. teysmannii* were 3,8-dihydroxy-1,2,4-trimethoxyxanthone (**3a**), 3-hydroxy-2,4-dimethoxyxanthone (**4**), 1,7-dihydroxy-3-methoxyxanthone (gentisin, **5**) (both listed in Peres and Nagem, 1997) and 2-hydroxyxanthone (**6b**, Sultanbawa 1980).

The ^{1}H NMR spectrum of xanthone **l**, $C_{16}H_{14}O_6$, exhibited signals of two sets of almost equivalent vicinally coupled aromatic protons and, in the ^{13}C NMR spectrum (Table 1), three methoxyls only one of which, at δ57.28, was adjacent to an unsubstituted carbon on the xanthone ring system. In fact proton and carbon chemical shifts in one of the rings were essentially identical with those in ring A of 1,8-dimethoxy-2-hydroxyxanthone we previously reported from this species (Gonzalez et al., 1999) while shifts of protons and carbons in the second ring were identical with those in ring A of 1,2-methoxy-8-hydroxyxanthone (ibid). The

☆ In honor of Professor Otto R. Gottlieb.

* Corresponding author. Fax: +351-22-2057358.
E-mail address: ankijjoa@icbas.up.pt (A. Kijjoa), jdulin@chem.fsu.edu (W. Herz).

0031-9422/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(00)00289-2

HMBC correlations depicted in Fig. 1 confirmed the assignment as 7-hydroxy-1,2,8-trimethoxyxanthone.

In xanthone **2** with the same empirical formula $C_{16}H_{14}O_6$, chemical shifts of protons and carbons in ring A paralleled those in ring A of xanthone **1** (see Table 1). The situation in ring B was, however, reversed with the methoxyl group at δ61.84 again located next to a hydroxyl group, but now at C-4. Structure **2**, 6-hydroxy-1,2,5-trimethoxyxanthone, was again confirmed by the HMBC correlations shown in Fig. 1.

A third constituent was a dihydroxytrimethoxyxanthone **3a** or **3b**. One of the hydroxyls was hydrogen bonded to the carbonyl group at C-9 (^{1}H NMR singlet at δ13.08) and attached to an otherwise unsubstituted aromatic ring as indicated by the ^{1}H and ^{13}C chemical shifts in ring B and the HMBC correlations shown in Fig. 1. However this technique was unable to distinguish between formulas **3a** and **3b**. We opted for formula **3a**, 3,8-dihydroxy-1,2,4-trimethoxyxanthone, because of the pronounced bathochromic shift in the UV spectrum on addition of base characteristic of a phenolic hydroxyl *para* to a carbonyl group.

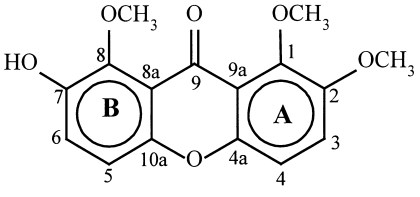
A xanthone assigned formula **3a** has been isolated previously from *Psorospermum febrigum* (Habib et al., 1987). Our ^{13}C NMR spectroscopy shifts in $CDCl_3$ (Table 1) differed only slightly from those reported earlier in DMSO; however, based on the HMBC correlations in Fig. 1 the shifts assigned to C-5 (111.7) and C-7 (104.0) by earlier workers probably need to be interchanged.

Xanthone **4** has been reported previously from two *Kielmeyera* (Gottlieb et al., 1970, 1971) and two *Hypericum* species (Luz Cardona et al., 1986, 1990); our ^{13}C NMR spectral data (Table 1) differ slightly from those reported previously (Castelão et a1., 1977) and were verified by the HMBC correlations depicted in Fig. 1.

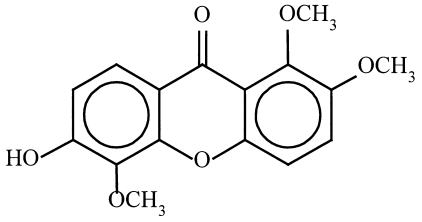
Xanthone **6a** appears to be new as a natural product although the literature contains two reports of its synthesis with little information provided on its properties (Eckstein et a1., 1983; Jackson et al., 1993). Mass spectrum and ^{1}H NMR data (see Section 3) indicated the presence of a carbomethoxy group in position 2 or 3 of ring A and a methoxy group in position 6 or 7 of ring B. The ^{13}C NMR spectrum (Table 1) supported these assignments while the HMBC correlations shown in Fig. 1 established the structure as 2-carbomethoxy-6-methoxyxanthone.

3. Experimental

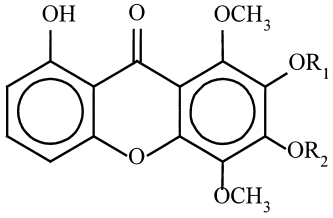
Extraction and fractionation of the extract of the wood of *C. teysmannii* var. *inophylloide* from Narathiwat Province, Southern Thailand, were described earlier (Gonzalez et al., 1999, Kijjoa et al., 2000). Frs 49-73 (0.7 g) of the first chromatogram were combined and rechromatographed (Si gel 60), 100 ml subfrs being collected as follows: Subfrs 1–5 (petrol–$CHCl_3$, 7:3), 6–94 (petrol–$CHCl_3$, 1:1), 95–109



1



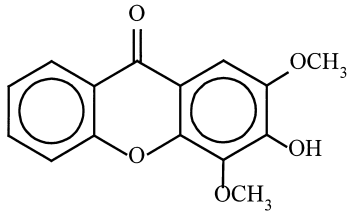
2



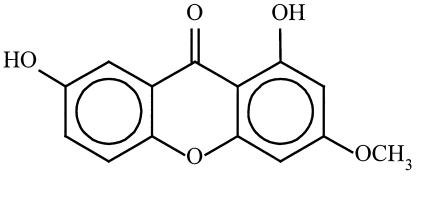
3

a - $R_1 = CH_3$, $R_2 = H$

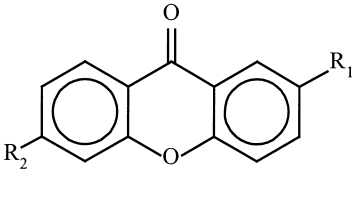
b - $R_1 = H$, $R_2 = CH_3$



4



5



6

a - $R_1 = CO_2Me$, $R_2 = OMe$

b - $R_1 = OH$, $R_2 = H$

Table 1
^{13}C NMR spectra of **1**, **2**, **3**, **4** and **6a** (75 MHz, $CDCl_3$)[a]

C	1	2	3	4	6a
1	148.76	149.09	131.26	100.60	129.24
2	148.88	149.45	148.44	144.81	126.01
3	120.29	119.59	149.04	145.07	134.96
4	113.14	112.94	137.75	134.67	118.15
4a	150.73	150.88	147.47	146.17	158.79
5	112.62	133.24	106.41	117.98	100.44
6	121.80	153.75	136.06	134.19	165.43
7	145.03	112.12	110.86	123.96	113.74
8	144.34	122.77	162.06	126.60	128.46
8a	116.22	116.87	108.66	121.34	115.72
9	176.02	175.61	181.52	176.16	175.63
9a	117.34	116.93	108.74	114.57	121.62
10a	150.19	148.99	155.91	155.89	157.92
1-OMe	61.73	61.70	62.10[b]		
2-OMe	57.28	57.12	61.80[b]	56.53	
3-OMe					
4-OMe			61.80[b]	61.73	
5-OMe		61.84			
6-OMe					55.93
8-OMe	62.54				
2-CO_2Me					165.99, 52.39

[a] Assignments of multiples by DEPT and HETCOR.
[b] Signals interchangeable.

(petrol–$CHCl_3$, 1:9), 160–170 ($CHCl_3$), 172–181 ($CHCl_3$–Me_2O, 4:1), 182–185 ($CHCl_3$–Me_2O, 3:2) and 186–197 ($CHCl_3$–Me_2O, 3:7). Purification of subfrs 12–16 by TLC (Si gel, $CHCl_3$–Me_2CO–HCO_2H, 80:20:1) gave **6a** (5 mg) and **3a** (15 mg). Purification of subfrs 17–31 by TLC (Si gel, toluene–EtOAc–HCO_2H, 65:35:1) gave 30 mg of 1,8-dimethoxy-2-hydroxyxanthone isolated previously from another fraction (Gonzalez et al., 1999), **1** (18 mg), **2** (16 mg), **4** (12 mg), an additional 12 mg of **6a**, **6b** (5 mg) and **5** (13 mg).

3.1. 7-Hydroxy-1,2,8-trimethoxyxanthone (*1*)

Brown solid, mp 73–75°C; HREIMS $[M]^+$ $m/z = 302.07901$ (calculated for $C_{16}H_{14}0_6$ $= 302.07905$); EIMS m/z (rel. int.) 302 [M]+ (100), 287 $[M–CH_3]^+$ (82.8), 272 (19.2), 256 (31.1), 244 (68.5), 227 (18.5), 201 (13.8), 149 (16.5); ^1H NMR (300 MHz, $CDCl_3$) δ7.31 (*d*, $J = 9.0$ Hz, H-6), 7.30 (*d*, $J = 9.2$ Hz, H-3), 7.14 (*d*, $J = 9.2$ Hz, H-4), 7.10 (*d*, $J = 9.2$ Hz, H-5), 4.01 (*s*, 3H, 8-OMe), 3.99 (*s*, 3H, 1-OMe), 3.90 (*s*, 3H, 2-OMe); ^{13}C NMR in Table 1; UV λ_{max}^{MeOH} nm (log ε) 386 (2.96), 265 (3.73), 242 (3.30); $\lambda_{max}^{MeOH+NaOH}$ nm (log ε) 254 (3.85), 210 (4.57); $\lambda_{max}^{MeOH+AlCl_3}$ no shift.

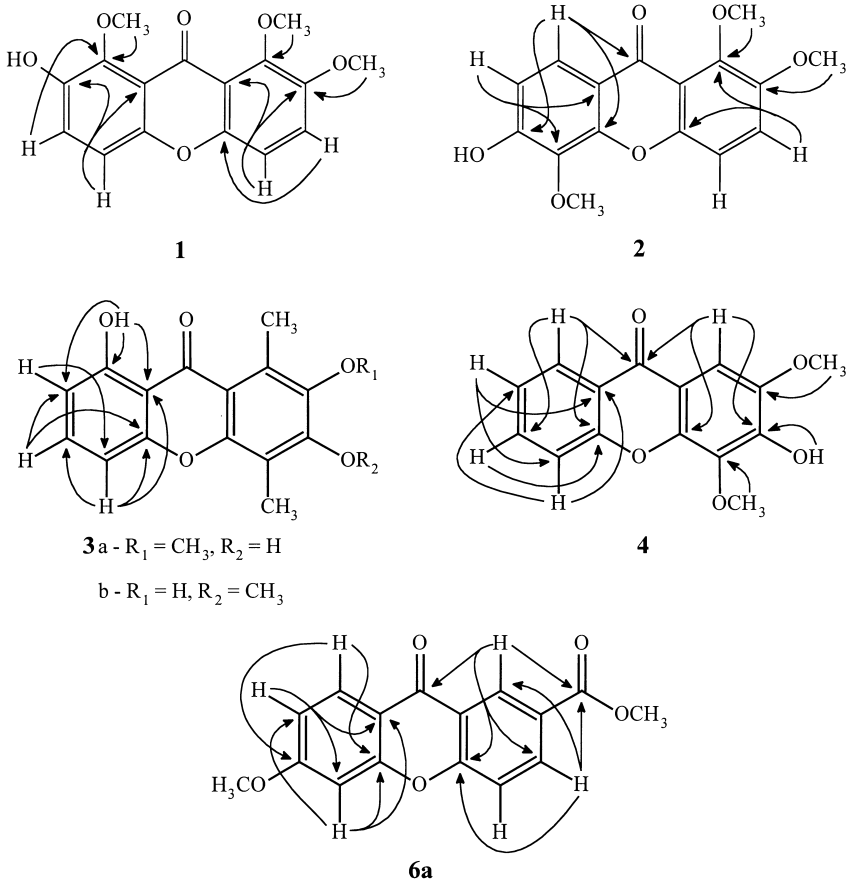


Fig. 1. HMBC of **1**, **2**, **3a**, **4** and **6a**.

3.2. 6-Hydroxy-1,2,5-trimethoxyxanthone (**2**)

Brown semisolid; HREIMS $[M]^+$ $m/z = 302.07907$ (calculated for $C_{16}H_{14}O_6 = 302.07904$); EIMS m/z (rel. int) 302 $[M^+]$ (100) 287 $[M–CH_3^+]$ (82.8), 272 (10.7), 256 (31.1), 244 (68.5), 149 (16.5); ^{1}H NMR (300 MHz, $CDCl_3$) δ7.96 (*d*, $J = 8.9$ Hz, H-8), 7.32 (*d*, $J = 9.2$ Hz, H-3), 7.28 (*d*, $J = 9.2$ Hz, H-4), 6.94 (*d*, $J = 8.9$ Hz, H-7), 4.10 (*s*, 3H, 5-OMe), 3.99 (*s*, 3H, 1-OMe), 3.90 (*s*, 3H, 2-OMe); ^{13}C NMR in Table 1; UV λ_{max}^{MeOH} nm (log ε) 349 (3.50), 310 (3.80), 284 (3.68), 242 (4.38); $\lambda_{max}^{MeOH+NaOH}$ nm (log ε) 358 (4.10), 238 (4.04), 209 (4.98); $\lambda_{max}^{MeOH+AlCl_3}$ no shift.

3.3. 3,8-Dihydroxy-1,2,4-trimethoxyxanthone (**3a**)

Mp 170–172°C, lit 182–184°C (Habib et al., 1987) HREIMS $[M]^+$ $m/z = 318.07401$ (calculated for $C_{16}H_{14}O_7 = 318.07395$); EI MS m/z (rel. int.) 318 $[M]^+$ (78.5), 303 $[M–CH_3^+]$ (100), 288 (13.7), 275 (48.5), 260 (45.9), 149 (48.2); ^{1}H NMR (300 MHz, $CDCl_3$) δ13.08 (*s*, 8-OH), 7.52 (*t*, $J = 8.3$ Hz, H-6), 6.91 (*d*, $J = 8.1$ Hz, H-5), 6.78 (*d*, $J = 8.3$ Hz, H-7), 4.03, 4.01, 3.97 (each *s* and 3H, OMes); ^{13}C NMR in Table 1; UV λ_{max}^{MeOH} nm (log ε) 314 (2.94), 2.51 (3.29); UV $\lambda_{max}^{MeOH+NaOH}$ nm (log ε) 380 (3.00), 238 (2.88), 209 (3.58); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε) 368 (3.11), 209 (3.90); $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm (log ε) 369 (2.97), 213 (3.93); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε) 345 (2.93), 281 (3.18), 263 (2.87), 202 (3.62); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε) 327 (2.90), 280 (3.18), 261 (3.27), 202 (3.63).

3.4. 2-Carbomethoxy-6-methoxyxanthone (**6a**)

Colorless crystals, mp 154–156°C; HREIMS $[M]^+$ $m/z =$ 284.06850 (calculated for $C_{16}H_{12}O_5 = 284.06847$); EIMS m/z (rel. int.) 284 $[M]^+$ (80.4), 271 (7.5), 253 (100), 225 (25.5), 197 (11.9), 182 (16.8), 149 (10.7), 126 (16.5); ^{1}H NMR (300 MHz, $CDCl_3$) δ8.98 (*d*, $J = 2.1$ Hz, H-1), 8.24 (*d*, $J = 9.0$ Hz, H-8), 7.71 (*dd*, $J = 8.6$, 1.2 Hz, H-7), 7.48 (*d*, $J = 8.8$ Hz, H-3), 6.96 (*dd*, $J = 9$, 2.5 Hz, H-4), 6.89 (*d*, $J = 2.3$ Hz, H-5), ^{13}C NMR in Table 1; λ_{max}^{MeOH} nm (log ε) 303 (3.61), 244 (4.13). Addition of NaOH and $A1C1_3$ caused no shift.

Acknowledgements

Work in Portugal was supported by Fundação para a Ciência e Tecnologia (Unidade de I&D No. 226/94) FEDER and PRAXIS XXI. We thank the Aveiro University Mass Spectrometry Group for low resolution mass spectra and Dr Graham Eaton, Department of Chemistry, University of Leicester, UK, for providing HRMS.

References

Cao, S.-G., Chang, K.L., Vittal, J.J., Sim, K.-Y., Goh, S.-H., 1998a. Isocalanone, a new pyranocoumarin from *Calophyllum teysmanii* (Guttiferae). Nat. Prod. Lett. 11, 233–236.

Cao, S.-G., Sim, K.-Y., Pereira, J., Goh, S.-H., 1996. Coumarins from *Calophyllum teysmannii*. Phytochemistry 47 (5), 773–777.

Cao, S.-G., Wu, X.Y., Sim, K.-Y., Tan, B.H.K., Vittal, J.J., Pereira, J.T., Goh, S.-H., 1998b. Minor coumarins from *Calophyllum teysmannii* var. *inophylloide* and synthesis of cytotoxic calanone derivatives. Helv. Chim. Acta 81, 1404–1416.

Castelão Jr, J.F., Gottlieb, O.R., DeLima, R.A., Mesquita, A.A.L., Gottlieb, H.E., Wenkert, E., 1977. Xanthonolignoids from *Kielmeyera* and *Caraipa* species-^{13}C NMR spectroscopy of xanthones. Phytochemistry 16, 735–740.

Eckstein, M., Marona, H., Mazur, J. 1983. Synthesis and some biological properties of substituted xanthone-2-carboxylic acids. Pol. J. Pharmacol. Pharm. 35, 159–167; (1984) Chem. Abstr. *100*, 6271 u.

Fuller, R.W., Bokesch, H.R., Gustafson, K.R., McKee, T.C., Cardellina II, J.H., McMahon, J.B., Cragg, G.M., Soejarto, D.D., Boyd, M.R., 1994. HIV-inhibitory coumarins from latex of the tropical rainforest tree *Calophyllum teysmannii* var. *inophylloide*. Bioorg. Med. Chem. Lett. 4, 1961–1964.

Gonzalez, M.J., Nascimento, M.S.J., Cidade, H.M., Pinto, M.M.M., Kijjoa, A., Anantachoke, C., Silva, A.M.S., Herz, W., 1999. Immunomodulatory activity of xanthones from *Calophyllum teysmannii* var. *inuphylloide*. Planta Medica 95, 368–371.

Gottlieb, O.R., Mesquita, A.A., Nagem, T.J., 1971. Xanthones from *Kielmeyera rubriflora*. Phytochemistry 10, 2253–2255.

Gottlieb, O.R., Mesquita, A.A., de Oliveira, G.G., de Mello, M.T., 1970. Xanthones from *Kielmeyera speciosa*. Phytochemistry 9, 2537–2544.

Gustafson, K.R., Bokesch, H.R., Fuller, R.W., Cardellina II, J.H., Kadushin, M.R., Soejarto, D.D., Boyd, M.R., 1994. Calanone, a novel coumarin from *Calophyllum teysmannii*. Tetrahedron Lett. 35, 5821–5824.

Habib, A.M., Reddy, K.S., McCloud, T.G., Chang, C.J., Cassidy, J.M., 1987. New xanthones from *Psorospermum febrifugum*. J. Nat. Prod. 50, 141–145.

Jackson, W.T., Boyd, R.J., Froelich, L.L., Gapinski, D.M., Mallett, E., Sawyer, J.S., 1993. Design, synthesis, and pharmacological evaluation of potent xanthone dicarboxylic acid leukotriene B4 receptor antagonists. J. Med. Chem. 36, 1726–1734.

Kijjoa, A., Gonzalez, M.J., Afonso, C.M., Pinto, M.M.M., Anantachoke, C., Herz W. 2000. Xanthones from *Calophyllum teysmannii* var. *inuphylloide*. Phytochemistry 53, 1021–1024.

Luz Cardona, M., Fernandez, I., Pedro, J.R., Seoane, E., Videal, R., 1986. Additional new xanthones and xanthonolignoids from *Hypericum canariensis*. J. Nat. Prod. 49, 95–100.

Luz Cardona, M., Fernandez, I., Pedro, J.R., Serrano, A., 1990. Xanthones from *Hypericum reflexum*. Phytochemistry 29, 3003–3006.

McKee, T.C., Covington, C.D., Fuller, R.W., Bokesch, H.R., Young, S., Cardellina II, J.H., Kadushin, M.R., Soejarto, D.D., Stevens, P.F., Cragg, G.M., Boyd, M.R., 1998. Pyranocoumarins from tropical species of the genus *Calophyllum*: a chemotaxonomic study of extracts in the National Cancer Institute collection. J. Nat. Prod. 61, 1252–1256.

McKee, T.C., Fuller, R.W., Covington, C.D., Cardellina II, J.H., Gulakowski, R.J., Krepps, B.L., McMahon, J.B., Boyd, M.R., 1996. New pyranocoumarins isolated from *Calophyllum lanigerum*and *Calophyllum teysmannii*. J. Nat. Prod. 59, 754–758.

Patil, A.D., Freyer, A.J., Eggleston, D.S., Haltiwanger, R.C., Bean, M.F., Taylor, P.B., Caranfa, M.J., Breen, A.L., Bartus, H.R., Johnson, R.K., Hertzberg, R.P., Westley, J.W., 1993. The inophyllums, novel inhibitors of HIV-1 reverse transcriptase isolated from malaysian tree, *Calophyllum inophyllum* Linn. J. Med Chem. 36, 4131–4138.

Peres, V., Nagem, T.J., 1997. Trioxygenated naturally occurring xanthones. Phytochemistry 44, 191–214.

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