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Xanthones from *Calophyllum inophyllum*

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ABSTRACT

The roots of *Calophyllum inophyllum* (Guttiferae), furnished six xanthones which are brasilixanthone (**1**), 1,3,5-trihydroxy-2- methoxy xanthone (**2**), caloxanthone A (**3**), pyranojacareubin (**4**), caloxanthone B (**5**) and tovopyrifolin (**6**), Structural elucidations of these compounds, were achieved through 1D and 2D NMR and MS techniques. In this paper, the isolation and structural elucidation data for these xanthones are reported.

Keywords: *Calophyllum inophyllum*, xanthones, NMR, MS

INTRODUCTION

Calophyllum inophyllum, which is also known as 'bintangor' by the locals in Malaysia, belongs to the Guttiferae family. Several species of this genus are known to be used in folk medicine (Dharmaratne and Wanigasekera, 1996). The wood has been used in general construction and boatbuilding, as well as for flooring, furniture, musical instruments, handicrafts, and a variety of other purposes (Shalan and Locksley, 1971). This genus has been found to be rich in xanthones (Iinuma *et al*., 1995; Kijoa *et al*., 2000; Ee *et al*., 2004*,* 2006), coumarins (Kashman *et al*., 1992; McKee *et al*., 1996) and flavonoids (Cao *et al*., 1997). Several coumarins isolated from two *Calophyllum* species were found to inhibit HIV-1 replication and cytopathicity activities (Pengsuparp *et al*., 1996; Kashman *et al*., 1992). The present work concentrates on the isolation of xanthones from the roots of *Calophyllum inophyllum.*

EXPERIMENT

Plant Material

The root bark of *Calophyllum inophyllum* was collected from UPM campus and identified by Dr. Rusea Go from the Department of Biology, UPM.

General

Infrared spectra were measured in KBr/NaCl pellet on a Perkin-Elmer FTIR Spectrum BX spectrometer. EIMS were recorded on a Shimadzu GCMS-QP5050A spectrometer. NMR spectra were obtained using a Unity INOVA 500MHz NMR/JEOL 400MHz FT NMR spectrometer, using tetramethylsilane (TMS) as an internal standard. UV spectra were recorded in $CHCl_3$ on a Shimadzu UV-160A, UV-Visible Recording Spectrophotometer. Melting points were measured using a leica Galen III microscope, equipped with Testo 720 temperature recorder.

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Extraction and Isolation

The dried roots of *Calophyllum inophyllum L.* (1.4 kg) were extracted with distilled n-hexane for 48 hours for three times. The combined n-hexane extract was concentrated under reduced pressure in a rotary evaporator. The same sample was then re-extracted twice each with distilled chloroform, followed by methanol for 48 hours. Eleven g of n-hexane extract, 60 g of chloroform extract and 21 g of methanol extract were obtained. The hexane, chloroform and methanol extracts were chromatographed through silica gel using hexane, chloroform, ethyl acetate and methanol as eluting solvents. The column chromatography on the crude hexane extract gave brasilixanthone **(1)** (5mg). 1,3,5-Trihydroxy-2-methoxy xanthone **(2)** (4 mg) and caloxanthone A **(3)** (15 mg) were obtained from the chloroform extract. Meanwhile, the methanol extract furnished pyranojacareubin **(4)** (4 mg), caloxanthone B **(5)** (6mg) and tovopyrifolin (**6**) (5 mg).

Brasilixanthone (1). Yellow crystals with m.p. 181-182ºC (Lit 181-182°C, Marques *et al*., 2000). UV ($CHCl_3$) λ_{max} nm: 317, 247. IR ν_{max} cm^{-1} (KBr): 3504, 2924, 1624, 1540, 1454, 1125, 1120, 750. EI-MS *m/z*: 392, 378, 377, 369, 347, 319, 279, 237, 203, 152, 115, 91, 77, 181 and 41. ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.62 (s, 1-OH,), δ 8.02 (d, 1H, *J*=10.0Hz, H-16), *δ* 6.83 (s, 1H, 6-OH), *δ* 6.72 (d, 1H, *J*=10.0Hz, H-12), δ 6.27 (s, 1H, H-5), *δ* 6.26 (s, 1H, H-4), *δ* 5.82 (d, 1H, *J*=10.0 Hz, H-17), *δ* 5.57 (d, 1H, *J*=10.0Hz, H-11), *δ* 1.47 (s, 6H, 14-CH_3, 15-CH_3), *δ* 1.50 (s, 6H, 19-CH_3, 20-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$):*δ* 156.5 (C-1), *δ* 103.9 (C-2), *δ* 153.0 (C-3), *δ* 94.3 (C-4), *δ* 157.8 (C-4a), *δ* 102.4 (C-5), *δ* 150.9 (C-6), *δ* 136.8 (C-7), *δ* 119.7 (C-8), *δ* 108.5 (C-8a), *δ* 182.5 (C-9), *δ* 104.4 (C-9a), *δ* 160.0(C-10a), *δ* 108.5 (C-11), *δ* 132.3 (C-12), *δ* 77.3 (C-13), *δ* 28.3 (C-14 & C-15), *δ* 120.9 (C-16), *δ* 127.2 (C-17), *δ* 77.3 (C-18), *δ* 27.3 (C-19 & C-20).

1,3,5-trihydroxy-2-methoxy xanthone (2). Pale yellow powder with m.p 182-184ºC (Lit 183-185°C, Iinuma *et al*., 1997). IR ν_{max} cm^{-1} (KBr): 3468, 1658, 1616, 1541, 1164, 1124, 854. EI-MS *m/z*: 274, 259, 231, 202, 187, 161, 147, 136, 107, 93, 77, 65, 51 and 41. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 13.11 (s, 1H, 1-OH), *δ* 9.48 (s, 1H, 5-OH), *δ* 9.26 (s, 1H, 3-OH), *δ* 7.64 (dd, 1H, *J* = 7.3, 1.8 Hz, H-8), *δ* 7.32 (dd, 1H, *J* = 8.2, 1.8 Hz, H-6), *δ* 7.26 (t, 1H, *J* = 15.6 Hz, H-7), *δ* 6.52 (s, 1H, H-4), *δ* 3.86 (s, 3H, 2-OMe). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 182.1 (C-9), *δ* 159.2 (C-3), *δ* 155.3 (C-1), *δ* 153.8 (C-4a), *δ* 146.9 (C-5), *δ* 146.0 (C-10a), *δ* 131.5 (C-2), *δ* 124.8 (C-7), *δ* 121.7 (C-8a), *δ* 121.3 (C-6), *δ* 116.1 (C-8), *δ* 103.9 (C-9a), *δ* 94.7 (C-4), *δ* 60.7 (2-OMe).

Caloxanthone A (3). Yellow needles with m.p. 136-138ºC (Lit 136-137°C, Iinuma *et al*., 1994). UV (EtOH) λ_{max} nm: 388, 494. IR ν_{max} cm^{-1} (KBr): 3516, 2982, 1650, 1612, 1650, 1450. EI-MS *m/z* : 394, 379, 323, 295, 281, 267, 237, 203, 175, 162, 148, 115, 105, 91, 77 and 55. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 13.89 (s, 1H, OH-1), *δ* 7.44 (s, 1H, H-8), *δ* 6.66 (d, *J* = 10.1Hz, 1H, H-11), *δ* 6.33 (s, 1H, H-4), *δ* 5.70 (d, *J* = 10.1Hz, 1H, H-12), *δ* 5.31 (t, *J* = 7.3Hz, 1H, H-17), *δ* 3.61 (d, *J* = 7.3Hz, 1H, H-16), *δ* 1.88 (s, 3H, C-19), *δ* 1.65 (s, 3H, H-20), *δ* 1.45 (s, 6H , H-14, H-15). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 180.8 (C-9), *δ* 160.6 (C-3), *δ* 158.3 (C-1), *δ* 158.0 (C-4a), *δ* 152.3 (C-10a), *δ* 150.8 (C-6), *δ* 143.4 (C-7), *δ* 132.5 (C-18), *δ* 128.5 (C-12), *δ* 122.4 (C-17), *δ* 116.6 (C-5), *δ* 115.9 (C-11), *δ* 113.2 (C-8a), *δ* 106.2 (C-8), *δ* 104.8 (C-2), *δ* 103.5 (C-9a), *δ* 95.3 (C-4), *δ* 78.7 (C-13), *δ* 28.4 (C-14), *δ* 28.4 (C-15), *δ* 25.8 (C-19), *δ* 23.0 (C-16), *δ* 18.1 (C-20).

Caloxanthone B (5). Yellow crystals with melting point 152-158ºC (Lit. 160.5ºC, Iinuma *et al*., 1994). UV (EtOH) λ_{max} nm: 317, 247. IR ν_{max} cm^{-1} (KBr): 3388, 2968, 1648, 1606, 1572, 1480, 1250. EI-MS *m/z*: 410, 395, 367, 352, 339, 337, 325, 176. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 13.75

(s, 1H, 1-OH), δ 7.28 (s, 1H, 6-OH), δ 6.84 (s, 1H, H-7), δ 6.26 (s, 1H, H-2), δ 5.38 (t, 1H, J=7.2Hz, H-17), δ 4.54 (q, 1H, J =6.6 Hz, H-14), δ 4.02 (s, 3H, 5-OCH_3), δ 4.01 (d, 2H, J=7.2 Hz, H-16), δ 1.78 (d, 3H, J=1.2Hz, 20-CH_3), δ 1.75 (s, 3H, 19-CH_3), δ 1.62 (s, 3H, 13-CH_3), δ 1.43 (s, 3H, 15-CH_3), δ 1.33 (s, 3H, 12-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.1 (C-9), δ 165.6 (C-3), δ 164.6 (C-1), δ 153.4 (C-6), δ 151.7(C-4a), δ 151.0 (C-10a), δ 142.1 (C-8), δ 133.3 (C-18), δ 132.2 (C-5), δ 122.3 (C-17), δ 113.3 (C-7), δ 112.1 (C-8a), δ 103.9 (C-9a), δ 94.0 (C-2), δ 90.6 (C-14), δ 61.9 (5-OCH_3), δ 33.6 (C-16), δ 25.9 (C-19), δ 25.6 (C-13), δ 21.6 (C-12), δ 18.0 (C-20), δ 14.2 (C-15).

Tovopyrifolin (**6**) Pale yellow crystals with m.p. 258-259ºC (Lit 256-258°C, Mesquita *et al*., 1975). UV (EtOH) λ_{max} nm: 313, 244, 217. IR ν_{max} cm^{-1} (KBr): 3468, 1584, 1464, 1218. EI-MS *m/z*: 274, 259, 231, 228, 136, 92. ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.11 (s, 1H, 1-OH), δ 9.48 (s, 1H, 3-OH), δ 9.26 (s, 1H, 5-OH), δ 7.64 (dd, 1H, J = 7.5Hz, 1.8 Hz, H-8), δ 7.34 (dd, 1H, J = 8.2Hz, 1.8 Hz, H-6), δ 7.28 (t, 1H, J = 7.5Hz, H-7), δ 6.52 (s, 1H, H-4), δ 3.86 (s, 3H, 2-OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$):δ 182.1 (C-9), δ 159.2 (C-3), δ 155.3 (C-1), δ 153.8 (C-4a), δ 146.9 (C-5), δ 146.1 (C-10a), δ 131.5 (C-2), δ 124.8 (C-7), δ 121.7 (C-8a), δ 121.3 (C-6), δ 116.1 (C-8), δ 104.0 (C-9a), δ 94.7 (C-4), δ 60.7 (OCH_3).

RESULTS AND DISCUSSION

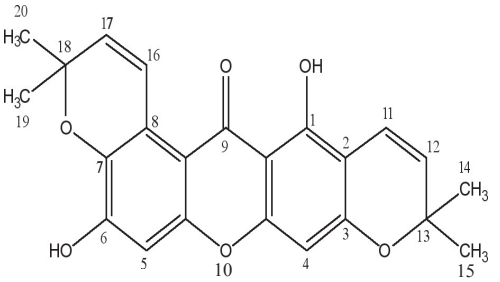
Pyranojacareubin **(4)**(10 mg) was obtained as fine yellow crystals with a m.p. of 185-189ºC (Lit. 187-189°C, Waterman and Crichton, 1980). The mass spectra data shows a molecular ion peak at *m/z* 392, indicating a molecular formula of $C_{23}H_{20}O_6$. The *m/z* peak of 377 was due to the loss of one methyl group, suggesting the existence of CH_3 as a side chain group.

The ^{1}H NMR spectrum showed the presence of one chelated hydroxyl group at δ 13.20. Two tall and sharp singlet peaks, at δ 1.36 and δ 1.41, which were integrated for 6 protons each, suggested four methyl groups. The COSY spectrum also indicated that the proton signal at δ 6.59 was coupled to the proton signal at δ 5.49. Meanwhile, the proton signal at δ 6.31 was coupled to the proton signal at δ 5.62. Thus, the four signals were assigned to the protons which were attached to C-11, C-12, C-16 and C-17. Four doublet peaks were observed at δ 6.59, δ 5.49, δ 6.31 and δ 5.62. These peaks have the same coupling constant value of 10.1 Hz, and the signals were assigned to H-11, H-12, H-16 and H-17, respectively.

The total number of carbon atoms observed in the ^{13}C NMR spectrum was 23. Meanwhile, the HMBC spectrum showed that two of the four methyl groups were attached to a similar carbon at C-13. Another two CH_3 groups were attached to C-18. The pyrano ring was obvious from the HMBC spectrum, indicating that the proton which was correlated to C-1 and C-3 was at C-11, and the proton at C-12 was correlated to C-2 via a ^3J coupling.

The HMBC spectrum also gave a ^2J correlation between the proton H-12 and C-13, while a ^3J correlation between proton H-11 and C-13 was observed. Meanwhile, proton H-11 had a correlation with C-12 and C-13 via a ^2J and ^3J coupling, respectively. On the other hand, the pyrano ring on the other side was obvious from the HMBC spectrum, which indicated that the proton at C-16 was correlated to C-7 via a ^2J coupling and the proton at C-17 was correlated to C-7 via a ^3J coupling. The HMBC spectrum also gave a ^3J correlation between proton H-16 and C-18.

From the DEPT spectrum, 6 C-H groups and no methylene groups were clearly seen in the compound. There was also evidence of 12 quarternary carbons and four CH_3 groups. Hence, compound **4** was identified to be pyranojacareubin previously isolated from *Garcinia densivenia* (Waterman and Crichton, 1980).



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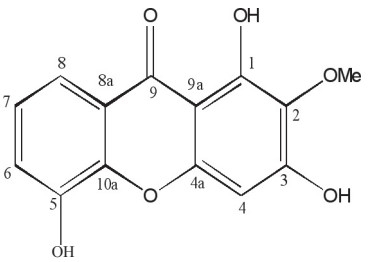

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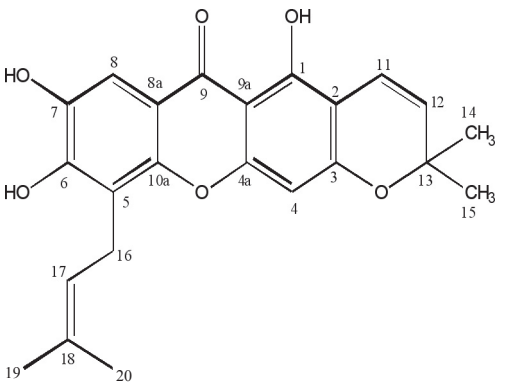

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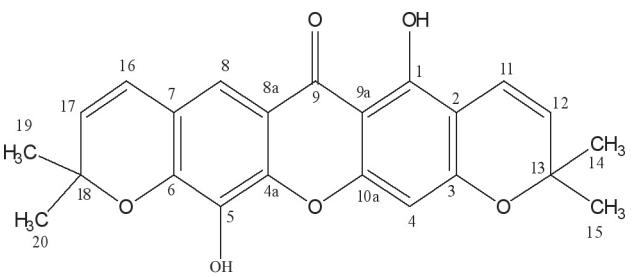

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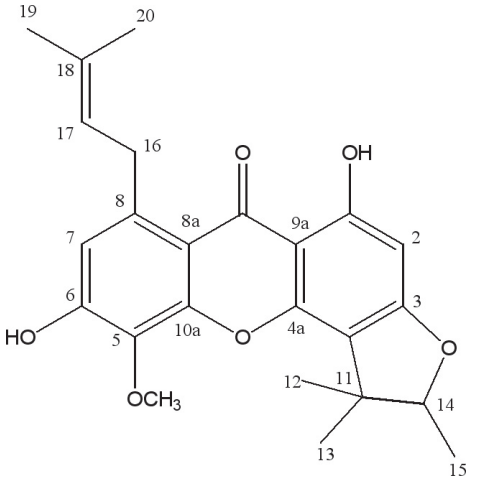

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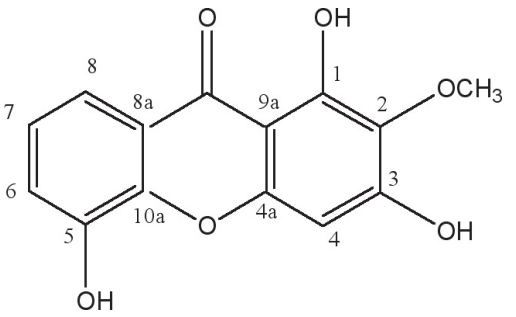

(6)

TABLE 1
^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$)
and HMBC assignments of Pyranojacareubin **(4)**

Position	δ_H	δ_C	HMBC
1	13.20 (OH)	157.7	-
2	-	104.8	-
3	-	160.4	-
4	6.30 (1H,s)	95.3	160.4 (C-3)
4a	-	156.8	-
5	-	132.1	-
6	-	144.8	-
7	-	117.8	-
8	7.30 (1H,s)	113.5	180.2 (C-9), 121.4 (C-16),
8a	-	114.6	-
9	-	180.2	-
9a	-	103.2	-
10a	-	145.1	-
11	6.59 (1H,d, J = 10.1Hz)	115.4	78.2 (C-13), 157.7 (C-1), 160.4 (C-3)
12	5.49 (1H, d, J = 10.1Hz)	127.5	78.2 (C-13), 104.8 (C-2)
13	-	78.2	-
14	1.36 (3H,s)	28.4	28.4 (C-15), 127.5 (C-12), 78.2 (C-13)
15	1.36 (3H,s)	28.4	28.4 (C-14), 127.5 (C-12), 78.2 (C-13)
16	6.31 (1H, d, J = 10.1Hz)	121.4	78.9 (C-18), 117.8 (C-7)
17	5.62 (1H,d, J = 10.1Hz)	131.0	78.9 (C-18), 117.8 (C-7)
18	-	78.9	-
19	1.41 (3H,s)	28.5	78.9 (C-18), 131.0 (C-17), 28.5 (C-20)
20	1.41 (3H,s)	28.5	78.9 (C-18), 131.0 (C-17), 28.5 (C-19)

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REFERENCES

Cao, S.G., Sim, K.Y. and Goh, S.H. (1997). Biflavonoids of *Calophyllum venulosum*. *Natural Product Letters*, *60,* 1245-1250.

Dharmaratne, H.R.W. and Wanigasekera, W.M.A.P. (1996). Xanthones from root bark of *Calophyllum thwaitesii*. *Phytochemistry, 42,* 249-250.

Ee, G.C.L., Kua, A.S.M., Cheow Y.L., Lim C.K., Jong V. and Rahmani, M. (2004). A new pyranoxanthone Inophyllin B from *Calophyllum inophyllum. Natural Product Sciences, 10*(5), 220-222.

Ee, G.C.L., Kua, A.S.M., Lim, C.K., Jong, V., Sukari, M.A. and Lee, H.L. (2006). Inophyllin A, a new pyranoxanthone from *Calophyllum inophyllum. Natural Product Research*, *20*(5), 485-491.

Iinuma, M., Tosa, H., Tanaka, T. and Yonemori, S. (1994). Two xanthones from roots of *Calophyllum inophyllum*. *Phytochemistry, 35,* 527-532.

Iinuma, M., Tosa, H., Tanaka, T. and Yonemori, S. (1995). Two xanthones from the roots of *Calophyllum inophyllum*. *Phytochemistry, 38,* 725-728.

Iinuma, M., Ito, T., Tosa, H., Tanaka, T., Miyake, R., and Chelladurai, V. (1997). Prenylated xanthonoids from *Calophyllum apetalum. Phytochemistry, 46,* 1423-1429.

Kashman, Y.K., Gustafson, R., Fuller, R.W., Cardellina, J.H., McMahon, J.B., Currens, M.J., Buckheit, R.W., Hughes, S.H., Cragg, G.M. and Boyd, M.R. (1992). The Calanolide, a novel HIV-Inhibitory class of coumarin derivatives from the tropical rainforest tree. *Calophyllum lanigerum*. *Journal of Medicinal Chemistry, 35,* 2735-2743.

Kijjoa, A., Jose, M., Gonzalez, T.G., Pinto, M.M., Damas, A.M., Mondranondra, I.O., Silva, A.M.S. and Herz, W. (2000). Xanthones from *Calophyllum teysimannii*. *Phytochemistry, 55,* 833-836.

Marques, V.LD., Oliveira, F.M., Conserva, L.M, Brito, R.G. and Guilhon, G.M. (2000). Dichromenoxanthones from *Tovomita brasiliensis*. *Phytochemistry, 55*(7), 815-818.

McKee, T.C., Richard, W.F., Conni, D.C., John, H.C. II, Robert, J.G., Benjamin, L.K., James, B.M. and Michael, R. (1996). New pyranocoumarins isolated from *Calophyllum lanigerum* and *Calophyllum teysmannii*. *Journal of Natural Product, 59,* 754-758.

Mesquita, A.A.L., Oliveira, W.G. and Neiva, R.M.T. (1975). Xanthones from *Tovomita pyrifolium*. *Phytochemistry, 14,* 803-806.

Pengsuparp, T., Serit, M., Hughes, S.H., Soejarto, D.D., and Pezzuto, J.M. (1996). Specific inhibition of human immunodeficiency virus type 1 reverse transcriptase mediated by soulattrolide, a coumarin isolated from the latex of *Calophyllum tesmannii. Journal of Natural Products, 59,* 839-842.

Shalan, F. and Locksley, H.D. (1971). Xanthones in the heartwood of *Calophyllum inophyllum*; A geographical survey. *Phytochemistry, 10*(3), 603-606.

Waterman, P.G. and Crichton, E.G. (1980). Xanthones and biflavanoids from *Garcinia densivenia* stem bark.