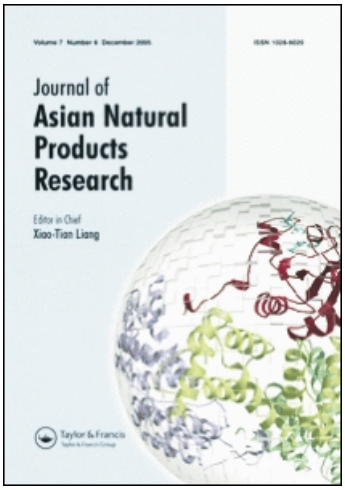


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new xanthones from *Artocarpus obtusus*

Najihah Hashim[a]; Mawardi Rahmani[a]; Mohd A. Sukari[a]; Abdul M. Ali[b]; Noorjahan B. Alitheen[b]; Rosea Go[c]; Hazar B. M. Ismail[d]
[a] Department of Chemistry, Universiti Putra Malaysia 43400 UPM, Selangor, Malaysia [b] Department of Cell and Molecular Biology, Universiti Putra Malaysia 43400 UPM, Selangor, Malaysia [c] Department of Biology, Universiti Putra Malaysia 43400 UPM, Selangor, Malaysia [d] Centre for Foundation Studies in Science, Universiti Malaya, Kuala Lumpur, Malaysia

Online publication date: 04 February 2010

To cite this Article Hashim, Najihah , Rahmani, Mawardi , Sukari, Mohd A. , Ali, Abdul M. , Alitheen, Noorjahan B. , Go, Rosea and Ismail, Hazar B. M.(2010) 'Two new xanthones from *Artocarpus obtusus*', Journal of Asian Natural Products Research, 12: 2, 106 — 112
To link to this Article: DOI: 10.1080/10286020903450411
URL: http://dx.doi.org/10.1080/10286020903450411

Journal of Asian Natural Products Research
Vol. 12, No. 2, February 2010, 106–112

ORIGINAL ARTICLE

Two new xanthones from *Artocarpus obtusus*

Najihah Hashim[a], Mawardi Rahmani[a]*, Mohd A. Sukari[a], Abdul M. Ali[b], Noorjahan B. Alitheen[b], Rosea Go[c] and Hazar B.M. Ismail[d]

[a]*Department of Chemistry, Universiti Putra Malaysia 43400 UPM, Serdang, Selangor, Malaysia;* [b]*Department of Cell and Molecular Biology, Universiti Putra Malaysia 43400 UPM, Serdang, Selangor, Malaysia;* [c]*Department of Biology, Universiti Putra Malaysia 43400 UPM, Serdang, Selangor, Malaysia;* [d]*Centre for Foundation Studies in Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*

(*Received 18 September 2009; final version received 26 October 2009*)

Two new xanthones, pyranocycloartobiloxanthone A (**1**) and dihydroartoindonesianin C (**2**), were isolated from the stem bark of *Artocarpus obtusus* Jarrett by chromatographic separation. Their structures were determined by using spectroscopic methods and comparison with known related compounds. Pyranocycloartobiloxanthone A (**1**) showed strong free radical scavenging activity by using DPPH assay as well as cytotoxicity towards K562, HL-60, and MCF7 cell lines.

Keywords: *Artocarpus obtusus*; Moraceae; pyranocycloartobiloxanthone A; dihydroartoindonesianin C; cytotoxicity

1. Introduction

The genus *Artocarpus* J.R. Forster and J.G. Forster (Moraceae), comprising about 55 species, is native to South and Southeast Asia, New Guinea, and the South Pacific [1]. Many of the species are cultivated for their edible fruit and some are used as material for light construction and furniture [1,2]. The bark, leaves, seeds, fruits, and roots of some species have medicinal properties and have been traditionally used in Southeast Asia for diarrhea [3,4]. Previous work on the genus revealed the occurrence of various phenolic compounds such as flavonoids containing isoprenyl substituents and certain oxygenation patterns [2,5]. Many of these compounds have been reported to show cytotoxic, antioxidant, and anti-inflammatory activity [6,7]. In this study, two new compounds, pyranocycloartobiloxanthone A (**1**) and dihydroartoindonesianin C (**2**), were isolated from the stem bark of the endemic and rare *Artocarpus obtusus* known locally as 'pala tupai'. In continuation of our study of bioactive compounds from tropical forest of Malaysia [8,9], we report the isolation of these new xanthone derivatives and their structural elucidation based on spectroscopic evidence and comparison with literature reports.

2. Results and discussion

A. obtusus was selected for further investigation on the basis of bioassay results. Extensive fractionation and purification of the crude $CHCl_3$ extract with a series of

*Corresponding author. Email: mawardi@fsas.upm.edu.my

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020903450411
http://www.informaworld.com

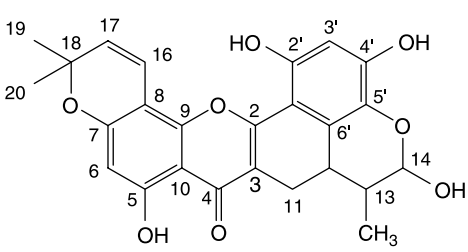
silica gel column chromatography and chromatotron yielded two new xanthone derivatives, pyranocycloartobiloxanthone A (**1**) and dihydroartoindonesianin C (**2**). Pyranocycloartobiloxanthone A (**1**) was obtained as yellow needle-shaped crystals, with mp 288–290°C. The HR-EI-MS of the compound displayed a molecular ion peak at *m/z* 450.1319, which supported the molecular formula $C_{25}H_{22}O_8$. With strong evidence from ^1H and ^{13}C NMR spectral data (Table 1), the molecular formula of the compound was further supported. The IR spectrum showed hydroxyl and conjugated carbonyl absorption bands at 3386 and 1650 cm^{-1}, respectively. The gross spectral features of the compound indicated a combination of structural relationship with artoindonesianin Z-2 (**3**) [2] and cycloartobiloxanthone (**4**) [10] with some differences. The NMR spectra were recorded in DMSO since the compound was relatively insoluble in other solvents. The ^1H NMR spectrum in the downfield region at δ 13.36 clearly indicated the typical characteristic signal of a chelated hydroxyl group (Table 1). Other signals in the ^1H NMR spectrum showed two singlets of the two chromene methyl groups at δ 1.40 and 1.42, together with the two doublets due to the olefinic protons at δ 6.86 and δ 5.74 ($J = 10.1$ Hz). This confirmed the presence of 2,2-dimethyl chromene [11]. The two aromatic singlets at δ 6.43 and δ 6.17 appeared similar to cycloartobiloxanthone (**4**) [10] and were assigned to protons H-3′ and H-6, respectively.

The methylene protons at C-11 occurred at two different chemical shifts δ 1.88 and 3.32 as triplet and doublet of doublet, respectively. These assignments are supported by the HMBC correlations of the protons to the neighboring carbon atoms (Table 1), and the DEPT spectrum clearly confirmed the existence of only one methylene group. The multiplet at δ 2.55 assigned to methine proton H-12 showed HMBC correlations with carbon signals at C-1′, C-6′, and C-3. The ^1H NMR spectrum also revealed the occurrence of an oxymethine proton at δ 5.32 as a multiplet and assigned to H-14, consistent with the presence of hemiacetal linkage [2]. Observation of the signal at δ 93.1 for the tertiary C-14 in the ^{13}C NMR spectrum supported the occurrence of this hemiacetal functionality. The tertiary methyl group at CH_3-15 occurred as a doublet at δ 1.08. The location of this group was determined by HMBC correlations with C-12, C-13, and C-14. Hence, based on these data and related literature values, the structure of pyranocycloartobiloxanthone A was established as that given in Figure 1, a hemiacetal flavone.

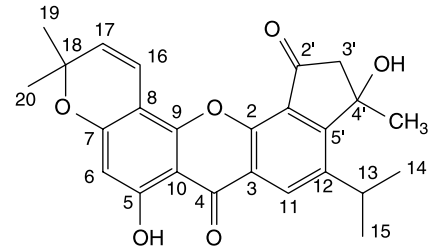
Dihydroartoindonesianin C (**2**) was obtained as yellowish needles, with mp 210–212°C. The HR-EI-MS spectrum gave a molecular ion peak at *m/z* 420.1560 for the molecular formula $C_{25}H_{24}O_6$ and further supported by the NMR spectral data (Table 2). The UV absorption bands at 288 (1.18), 345 (0.40), and 394 (0.16) nm are consistent with the existence of a xanthone skeleton and the IR spectrum supports the occurrence of hydroxyl, chelated hydroxyl, two carbonyl (1728 and 1654 cm^{-1}), and aromatic ring functionalities. The spectral data of the compound were compared with those of artoindonesianin C (**5**) [12], previously isolated from *Artocarpus tysmanii* Mig. which showed some similarities and differences (Table 2). The ^1H NMR spectral data showed the occurrence of a sharp methyl singlet at δ 1.76 (six protons) and two olefinic doublets at δ 5.65 and 6.61, each with a coupling constant of 10.3 Hz, typical characteristic of a 2,2-dimethylchromene ring [11]. Only two aromatic protons were observed in the spectrum, and they occurred as singlets at δ 8.32 and 6.32 and are attributed to H-11 and H-6, respectively. Other characteristics of the spectrum included signals for a chelated hydroxyl (δ 12.50), a tertiary hydroxyl (δ 4.22), and four oxyaril (δ 151.1, 151.2, 163.2, and 166.7) groups, suggesting that compound **2** contains oxygen functionalities at C-2, C-9, C-7, and C-5. The DEPT

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1**, **3**, and **4**.

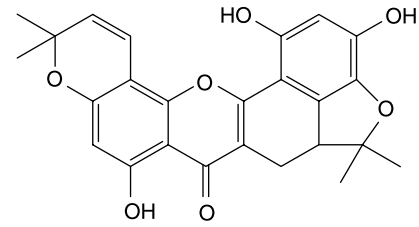
	1 in DMSO-d_6			**3** in DMSO-d_6 [2]		**4** in acetone-d_6 [10]	
H/C	δ_H	δ_C	HMBC	δ_H	δ_C	δ_H	δ_C
2	–	160.7	–	–	160.5	–	162.8
3	–	100.8	–	–	111.2	–	102.1
4	–	174.2	–	–	178.9	–	181.9
5	–	151.2	–	–	161.3	–	152.4
6	6.17 (1H, s)	98.7	C-10, 8, 7	6.14	98.71	6.14	102.2
7	–	160.7	–	–	163.4	–	163.1
8	–	104.3	–	6.30	93.6	–	105.2
9	–	157.9	–	–	156.6	–	158.6
10	–	103.6	–	–	103.4	–	105.2
11	1.88 (1H, t, $J = 5.2$ Hz)	21.5	C-2, 12	1.82	21.7	2.36	20.5
	3.32 (1H, dd, $J = 5.2$, 6.0 Hz)		C-2, 3, 12	3.11		3.43	
12	2.55 (1H, m)	35.7	C-1′, 6′, 3	2.53	35.7	3.20	47.7
13	1.82 (1H, m)	31.2	–	1.76	31.3	–	93.9
14	5.32 (1H, m)	93.1	–	5.30	93.1	1.67	22.9
15	1.08 (3H, d, $J = 7.3$ Hz)	14.6	C-12, 13, 14	1.06	14.6	1.34	29.1
16	6.86 (1H, d, $J = 10.1$ Hz)	114.8	C-18, 17	–	–	6.92	116.3
17	5.74 (1H, d, $J = 10.1$ Hz)	127.3	C-18	–	–	5.64	128.2
18	–	78.0	–	–	–	–	78.9
19	1.40 (3H, s)	27.7	C-18, 17	–	–	1.47	28.3
20	1.42 (3H, s)	28.0	–	–	–	1.47	28.3
1′	–	111.0	–	–	103.9	–	113.0
2′	–	151.1	–	–	150.8	–	151.9
3′	6.43 (1H, s)	103.1	C-5′, 4′, 2′	6.37	103.2	6.43	106.8
4′	–	150.8		–	150.8	–	147.8
5′	–	132.5	–	–	132.4	–	138.6
6′	–	124.6	–	–	124.7	–	134.0
OH-5	13.36 (1H)		–	13.24		13.33	
OH-7	–			9.44		–	
OH-14	6.93 (1H)		–	6.88		–	
OH-2′	9.91 (1H)		–	9.85		8.70	
OH-4′	9.81 (1H)		C-4′, 3′	10.63		8.85	



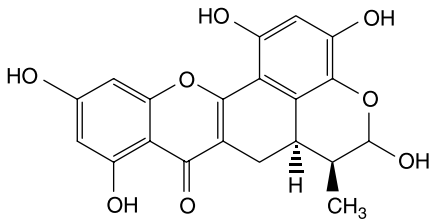
1



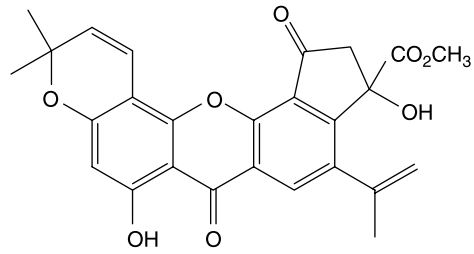
2



4



3



5

Figure 1. The structures of compounds **1**–**5**.

spectrum clearly indicated the presence of 25 carbon atoms including five methyls, one methylene, five methines, and two carbonyls (δ 179.1 and 198.4). Hence, the basic skeleton of the molecule contains a chromene ring, a fused xanthone ring, and a five-membered ketone ring. The isopropre-nyl side chain in **5** was replaced by an isopropyl substituent as collaborated by the appearance of a six proton doublet for the two methyl groups at δ 1.50. The methine proton H-13 and the methylene protons H-3′ overlapped each other at δ 2.81. All of the above supportive evidence are

Table 2. ^{1}H and ^{13}C NMR spectral data and selected HMBC correlations for dihydroartoindonesianin C (**2**) and artoindonesianin C (**5**).

	2 in $CDCl_3$			**5** in DMSO-d_6 [12]	
H/C	δ_H	δ_C	HMBC	δ_H	δ_C
2	–	151.1	–	–	150.1
3	–	119.2	–	–	120.7
4	–	179.1	–	–	178.9
5		166.7	–	–	162.1
6	6.32 (1H, s)	100.1	C-10, 7	6.30	99.1
7	–	163.2	–	–	160.6
8	–	101.4	–	–	101.1
9	–	151.2	–	–	150.9
10	–	103.5	–	–	103.5
11	8.32 (1H, s)	128.2	C-2, 4	8.15	131.3
12	–	148.6	–	–	141.0
13	2.81 (1H, m)	130.2	–	–	137.6
14	1.50 (3H, d, $J = 7.6$ Hz)	28.0	C-12, 13	5.02/5.25	131.3
15	1.50 (3H, d, $J = 7.6$ Hz)	28.0	C-12, 13	2.10	24.5
16	6.61 (1H, d, $J = 10.3$ Hz)	114.2	C-8	6.89	113.8
17	5.65 (1H, d, $J = 10.3$ Hz)	126.5	C-8	5.90	128.4
18	–	86.6	–	–	78.9
19	1.76 (3H, s)	29.7	C-18	1.46	28.0
20	1.76 (3H, s)	29.7		1.46	28.0
1′	–	125.1	–	–	124.3
2′	–	198.4	–	–	198.0
3′	2.81 (2H, s, $-CH_2$)	32.3	–	2.92/3.28	52.6
4′		79.1	–	–	76.3
5′	–	162.3	–	–	158.6
6′	1.25 (3H, s, $-CH_3$)	27.6	–	–	172.6
OH-5	12.50 (1H, br, $-OH$)	–		12.58	
OH-4′	4.22 (1H, br, $-OH$)	–		6.78	
OMe	–			3.60	

in agreement with the structure of compound (**2**), with the trivial name dihydroartoindonesianin C.

The DPPH assay described by Lee *et al.* [13] was used to show that compound **1** is a strong free radical scavenger with an IC_{50} value of 2.0 μg/ml. This compound also exhibited strong cytotoxic activity against K562, HL-60, and MCF7 cell lines with IC_{50} values of 0.5, 2.0, and 5.0 μg/ml, respectively.

3. Experimental

3.1 General experimental procedures

All melting points (mp) were determined using a hot-stage melting-point apparatus model Leica Galen III equipped with microscope and are uncorrected. UV and IR spectra were measured with Shimadzu UV 2100 and Perkin Elmer FT-IR (model 1725X) spectrophotometers, respectively. The ^{1}H and ^{13}C NMR spectra were obtained with JEOL ECA-400 spectrometer operating at 400 and 100 MHz with tetramethylsilane (TMS) as the internal standard, respectively. The MS were obtained with a Shimadzu GCMS-QP5050 spectrometer with a direct induction probe (DIP) using ionization induced by electron impact at 70 eV. Column chromatography was packed with Merck silica gel 60 (0.063–0.200 mm mesh size; Darmstadt, Germany). Analytical thin layer chromatography (TLC) was performed on commercially available Merck TLC plastic sheets precoated with Keisegel 60 F_{254}, 0.2 mm thickness, and the

chromatotron plates were coated with Keisegel 60 PF_{254} and scrapped to 0.75 mm thickness.

3.2 Plant material

Samples of the air-dried stem bark were collected from Sarawak in 2004. A voucher specimen (S94402) has been deposited at the Herbarium, Department of Biology, Faculty of Science, Universiti Putra Malaysia and identified by Dr Rosea Go of the department.

3.3 Extraction and isolation

The dried and ground stem bark (640 g) was sequentially extracted with *n*-hexane, $CHCl_3$, and MeOH at room temperature. The extracts were concentrated *in vacuo*. The crude $CHCl_3$ extract (9 g) was coated on silica gel and fractionated by gravity column chromatography. Solvents of increasing polarity consisting of petroleum ether (PE), PE–$CHCl_3$, $CHCl_3$, $CHCl_3$–EtOAc, EtOAc–MeOH, and MeOH were used to elute 36 fractions of 200 ml each. The combined fractions 21–23 were further purified by column chromatography, and the yellow solid obtained was washed with MeOH and recrystallized from DMSO to give pyranocycloartobiloxanthone A (**1**) as yellow needle-shaped crystals (67.8 mg). The mother liquor was further chromatographed twice using silica gel and eluted with *n*-hexane with gradually increasing proportions of $CHCl_3$ and MeOH. The combined fractions 9–14 were then separated by chromatotron and eluted with *n*-hexane–EtOAc (88:12) to afford a yellow solid of dihydroartoindonesianin C (**2**), which was recrystallized with DMSO to give needle-shaped crystals (8 mg).

3.3.1 Pyranocycloartobiloxanthone A (*1*)

Yellow needles (67.8 mg), mp 288–290°C; IR (KBr) ν_{max}: 3386 (OH), 2928 (saturated C—H), 1650 (C═O), 1606, 1552, 1480, 1362, 1276, 1154, 994 cm^{-1}; UV (DMSO) λ_{max} (log ε): 228 (1.25), 275 (1.35), 309.5 (0.47), 394.5 (0.99) nm; ^1H NMR (DMSO-d_6, 400 MHz) spectral data, see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz) spectral data, see Table 1; EI-MS *m/z* 450 $[M]^+$(35.66), 435 (100), 377 (55.74), 347 (13.86), 321 (5.82), 279 (4.25), 203 (10.07), 189 (44.81), 166 (24.85); HR-EI-MS *m/z*: 450.1319 $[M]^+$ (calcd for $C_{25}H_{22}O_8$, 450.1315).

3.3.2 Dihydroartoindonesianin C (*2*)

Yellow needles (8 mg), mp 210–212°C; IR ($CHCl_3$) ν_{max}: 3384 (OH), 2920 (saturated C—H), 1728, 1654, 1595, 1460, 1018 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε): 288 (1.18), 345 (0.40), 394 (0.16) nm; ^1H NMR ($CDCl_3$, 400 MHz) spectral data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) spectral data, see Table 2; EI-MS *m/z* 420 (10.47) $[M]^+$, 406 (26.55), 405 (100), 334 (4.55), 195 (10.06), 174 (8.29); HR-EI-MS *m/z*: 420.1560 $[M]^+$ (calcd for $C_{25}H_{24}O_6$, 420.1566).

Acknowledgements

We wish to thank Universiti Putra Malaysia for providing laboratory facilities; the Malaysian Government for providing a research grant under Intensification of Research in Priority Areas (IRPA) Program; and one of us (NH) wishes to thank Universiti Sains Malaysia for granting study leave.

References

[1] K.M. Kochummen and R. Go, *Tree Flora Sabah and Sarawak* (Ampang Press Sdn. Bhd, Kuala Lumpur, 2000), Vol. 3, p. 181.
[2] E.H. Hakim, S.A. Achmad, L.D. Juliawaty, L. Makmur, Y.M. Syah, N. Aimi, M. Kitajima, H. Takayama, and E.L. Ghisalberti, *J. Nat. Med.* **60**, 161 (2006).
[3] Y. Wang, K. Xu, L. Lin, Y. Pan, and X. Zheng, *Phytochemistry* **68**, 1300 (2007).

[4] B.N. Su, M. Cuendet, M.E. Hawthorne, L.B.S. Kardono, S. Riswan, H.H.S. Fong, R.G. Mehta, J.M. Pezzuto, and A.D. Kinghorn, *J. Nat. Prod.* **65**, 163 (2002).
[5] T. Nomura and Y. Hano, *Nat. Prod. Rep.* **11**, 205 (1994).
[6] Y.H. Wang, A.J. Hou, L. Chen, D.F. Chen, H.D. Sun, O.S. Zhao, K.F. Bastow, Y. Nakanishi, X.H. Wang, and K.H. Lee, *J. Nat. Prod.* **67**, 757 (2004).
[7] S.A. Achmad, E.H. Hakim, L.D. Juliawaty, L. Makmur, and Y.M. Syah, *Malaysian J. Sci.* **24**, 7 (2005).
[8] M. Rahmani, R.A. Susidarti, H.B.M. Ismail, M.A. Sukari, Y.H. Taufiq-Yap, G.C.L. Ee, A.M. Ali, J. Kulip, and P.G. Waterman, *Phytochemistry* **64**, 873 (2003).
[9] I. Komala, M. Rahmani, M.A. Sukari, H.B.M. Ismail, G.C.L. Ee, and A. Rahmat, *Nat. Prod. Res.* **20**, 355 (2006).
[10] M.U.S. Sultanbawa and S. Surendrakumar, *Phytochemistry* **28**, 599 (1989).
[11] C.N. Lin, W.L. Shieh, and T.T. Jong, *Phytochemistry* **31**, 2563 (1992).
[12] L. Makmur, Syamsurizal, Tukiran, S.A. Achmad, N. Aimi, E.H. Hakim, M. Kitajima, and H. Takayama, *J. Nat. Prod.* **63**, 243 (2000).
[13] S.K. Lee, H.M. Zakaria, H.S. Chung, L. Luyengi, E.J.C. Gamez, R. Mehta, A.D. Kinghorn, and J.M. Pezzato, *Combinant Chem. High Throughput Screen* **1**, 35 (1998).