Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 569-572

ASIAN JOURNAL OF CHEMISTRY

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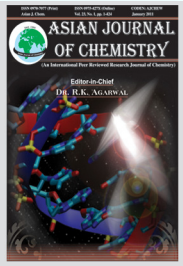

Cratoxylum glaucum and *Cratoxylum arborescens* (Guttiferae)- Two Potential Source of Antioxidant Agents

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(*Received*: 8 February 2010; *Accepted*: 20 September 2010) AJC-9110

Our detailed chemical studies on *Cratoxylum glaucum* and *C. arborescens* have revealed the presence of 5'-demethoxycadensin G (**1**), fuscaxanthone C (**2**), β-mangostin (**3**), 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone (**4**), vismiaquinone (**5**), 1,8-dihydroxy-3-methoxy-6-methylanthraquinone (**6**), stigmasterol (**7**) and friedelin (**8**). Structural elucidations of these compounds were achieved by using 1D and 2D NMR spectroscopic experiments. Antioxidant tests conducted on these two plant species gave promising results with both species indicating good antioxidant inhibiting properties. This is a first report on 5'-demethoxycadensin G (**1**) and β-mangostin (**3**) from *Cratoxylum glaucum* as well as the antioxidant properties of these two species.

Key Words: *Cratoxylum arborescens*, *Cratoxylum glaucum*, Guttiferae, Xanthone, Anthraquinone, Antioxidant.

INTRODUCTION

The genus *Cratoxylum* from the Guttiferae family consists of six species and is mainly distributed in the Southeast Asian region[1]. *Cratoxylum* is sometimes categorized under the family Hypericaceae. The stem bark of the species usually exudes a resin and has been applied in traditional medicine by the local people of Malaysia[2]. Reports indicated that the bark, roots and leaves have been used in folk medicine to treat fevers, cough, diarrhoea, itches, ulcers and abdominal complaints[3]. There are few phytochemical reports on the *Cratoxylum* species. However, some investigations on the phytochemistry of this genus have revealed it to be rich in flavonoids, xanthones and triterpenoids[2,4-6]. Antibacterial, cytotoxic and anti HIV constituents have also been reported in recent studies on *Cratoxylum* species[7,8]. The isolation and identification of 5'-demethoxycadensin G (**1**) from *Cratoxylum glaucum* and the antioxidant properties of *Cratoxylum arborescens* and *C. glaucum* are reported in this paper.

EXPERIMENTAL

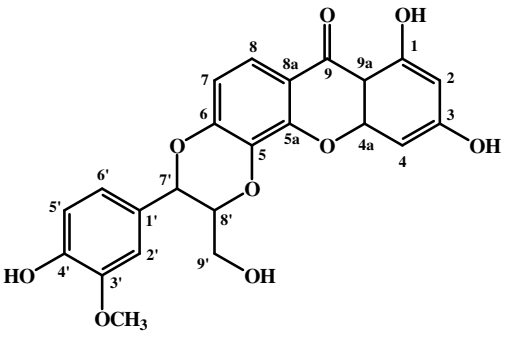
The stem bark of *Cratoxylum arborescens* and *C. glaucum* were collected from Sri Aman, Sarawak, Malaysia. The plant materials were identified by Dr. Rusea Go of Biology Department, UPM, Malaysia.

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 400 MHz FT NMR spectrometer using tetramethylsilane as the internal standard. Ultra violet spectra were recorded in $CHCl_3$ on a Shimadzu UV-160A, UV-visible recording spectrometer.

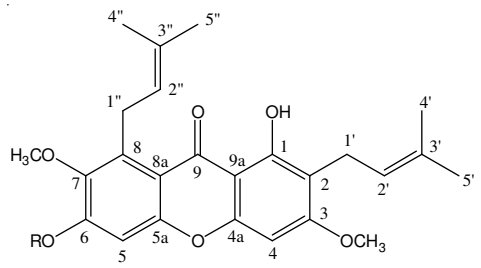
Extraction and isolation: The air-dried and powdered stem bark of *Cratoxylum glaucum* (3.75 kg) was extracted successively with hexane, ethyl acetate, methanol and acetone at room temperature. The extracts were evaporated to dryness under reduced pressure to yield 26 g of hexane extract, 136 g of ethyl acetate extract, 49 g of methanol extract and 20 g of acetone extract. The extracts were subjected to a series of column chromatography over silica gel columns using a stepwise gradient system (hexane/ethyl acetate and ethyl acetate/methanol). The eluents were collected in a volume range of 50-250 mL depending on the size of the column used. The column chromatography of the hexane extract of both plant species gave fuscaxanthone C (**2**), stigmasterol (**7**) and friedelin (**8**). The chloroform extract of *Cratoxylum arborescens* gave vismiaquinone (**5**) while the ethyl acetate extract of *Cratoxylum glaucum* afforded 5'-demethoxycadensin (**1**), β-mangostin (**3**), 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone (**4**), 1,8-dihydroxy-3-methoxy-6-methylanthraquinone (**6**).

Fuscaxanthone C (2): Yellow crystals, m.p. 114-115 °C (lit.[9] 110-116 °C). UV (EtOH) λ_{max} nm (log ε): 246.5 (4.40), 263.5 (4.47), 313.0 (4.30), 351.5 (3.75); IR (KBr, ν_{max}, cm^{-1}): 3390 (broad OH), 2861 (C-H stretching), 1643 (C=O), 1598 (C=C), 1458 (C=C), 1379 (CH_3 bending), 1286 (C-CO-C);

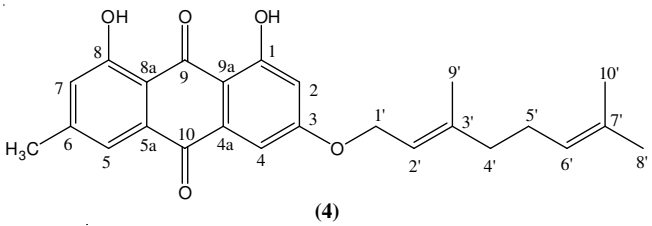
EI-MS m/z (rel. int.): 438 (100), 423 (6), 395 (57), 367 (74), 351 (29), 339 (36), 313 (21), 191 (15), 176 (31); ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR: spectral data are in agreement with literature[10].



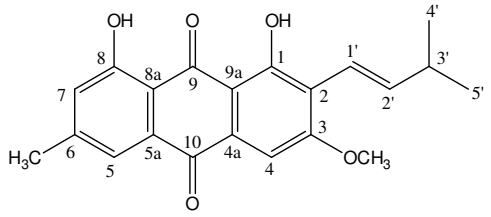
(**1**)



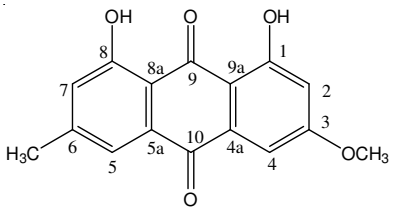
(**2**): R = CH_3; (**3**): R = H



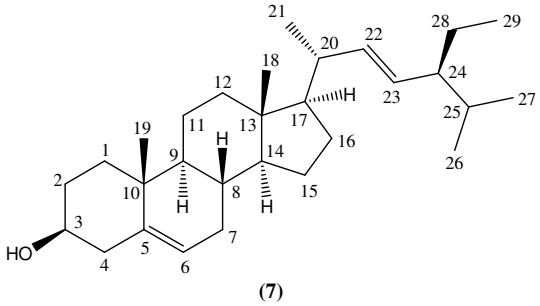
(**4**)



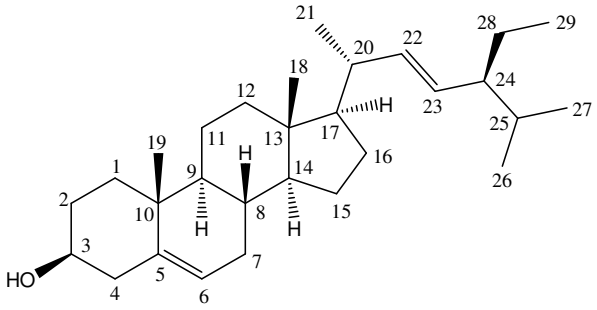
(**5**)



(**6**)



(**7**)



(**8**)

β-Mangostin (3): Orange crystals, m.p. 170-171 ºC (lit.[11] 175-176 °C). UV (EtOH) λ_{max} nm (log ε): 246 (4.45), 261 (4.50), 313 (4.33), 355 (3.78); IR (KBr, ν_{max}, cm^{-1}): 3395 (broad OH), 2922 (C-H stretching), 1646 (C=O), 1597 (C=C), 1456 (C=C), 1378 (CH_3 bending), 1282 (C-CO-C); EI-MS m/z (rel. int.): 424 (37), 381 (20), 368 (42), 353 (100), 335 (35); ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR spectral data are in agreement with published data[11].

3-Geranyloxy-6-methyl-1,8-dihydroxyanthraquinone (4): Orange crystals, m.p. 119-120 °C (lit.[12] 119-121 °C). UV (EtOH) λ_{max} nm (log ε): 246 (4.45), 261 (4.50), 313 (4.33), 355 (3.78); IR (KBr, ν_{max}, cm^{-1}): 3438 (broad, OH), 2928 (C-H), 1626 (C=O), 1480 (C=C), 1392 (C=C); EI-MS m/z (rel. int.): 406 (11), 378 (2), 363 (4), 335 (2), 316 (5), 295 (12), 270 (14); ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR spectral data are in agreement with literature[12].

Vismiaquinone (5): Orange crystals, m.p. 200-202 ºC (lit.[13] 202-204 °C). UV (EtOH) λ_{max} nm (log ε): 250.5 (3.90), 265.5 (3.93), 295.0 (4.09), 446.5 (3.78); IR (KBr, ν_{max}, cm^{-1}): 3438 (broad OH), 2946 (C-H stretch.), 2852 (C-H stretch.), 1624 (C=O), 1557 (C=C), 1476 (C=C), 1378 (CH3 bending), 1290 (C-CO-C); EI-MS m/z (rel. int.): 352 (36), 337 (13), 323 (4), 309 (100), 297 (28), 283 (5), 267 (6), 161 (10); ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR spectral data are in agreement with literature[14].

1,8-Dihydroxy-3-methoxy-6-methylanthraquinone (6): Light orange crystals, m.p. 198-199 °C (lit.[11] 175-176 °C). UV (EtOH) λ_{max} nm (log ε): 2.42 (3.90), 274 (4.11), 445 (3.73); IR (KBr, ν_{max}, cm^{-1}): 3440 (broad OH), 1633 (C=O), 1567 (C=C), 1471 (C=C), 1372 (CH_3 bending); EI-MS m/z (rel. int.): 284 (100), 255 (17), 241 (15), 241 (18), 227 (9), 213 (16), 198 (9), 128 (25); ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data are in agreement with published data[14].

Determination of antioxidant activity: Antioxidant properties of these two *Cratoxylum* species extracts were evaluated based on their free-radical scavenging activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical. This assay was carried out based on the method described by Wang *et al.*[15] and Molyneux[16] with slight modifications. Ethanolic solutions of extracts were prepared in a series of concentration (*e.g.* 5, 10, 15, 20, 25, 30, 35 ppm). 2 mL of each solution were added to 2 mL of 0.2 mM DPPH solution. The mixtures were kept in the dark for 0.5 h before their absorbance at 517 nm were measured using a spectrophotometer. Their antioxidant activities were then compared with that of ascorbic acid.

Determination of total phenolic content: The total phenolic content of extracts was measured using Folin-Ciocalteu method as applied by Chew *et al.*[17]. Standard methanolic solutions of the crude extract (1 mg/mL) were prepared and suitable dilutions were carried out with H_2O. 1.5 mL Folin-Ciocalteu's phenolic reagent (10 % v/v) and 1.2 mL 7.5 % w/v Na_2CO_3 were added to 0.3 mL of the diluted extract solution. The mixture was kept in the dark at room temperature for 0.5 h before its absorbance was measured at 765 nm in a UV-vis spectrophotometer. Total phenolic content is expressed in terms of mg gallic acid equivalent (GAE) per mg of extract.

RESULTS AND DISCUSSION

5'-Demethoxycadensin G (**1**) was isolated as pale yellow crystals with melting point 278.1-278.8 °C. The EIMS gave an $[M^+]$ ion peak at 438 consistent with a molecular formula $C_{23}H_{18}O_9$. The IR spectrum indicated the presence of O-H stretching at 3506 cm^{-1}, aromatic C-H stretching at 3392 cm^{-1} and alkane C-H stretching at 2934 cm^{-1}, C=O stretching at 1646 cm^{-1}, aromatic C=C stretching at 1614 cm^{-1} and C-O stretching at 1172 cm^{-1}. The signal at 1338 cm^{-1} is due to the $-CH_3$ and aromatic C-H (out-of-plane) bending at 912, 818 and 766 cm^{-1}.

The ^1H NMR spectrum related to the presence of a hydroxyl group at δ 12.95 which is attached to C-1. Seven aromatic proton signals were observed at δ 7.59 (d), 7.03 (t), 7.04 (d), 6.89 (dd), 6.82 (d), 6.39 (d) and 6.20 (d). The pair of *meta* coupled doublets at δ 6.20 and 6.39 were assigned to C-2 and C-4, respectively. The *ortho* coupled doublet at δ 6.82 (J = 8.3 Hz) and the doublet of doublet (*ortho* and *meta* coupled) signal at δ 6.89 (J = 8.3, 1.8 Hz) were assigned to H-5' and H-6', respectively. Meanwhile, the doublet signal at δ 7.04 (J = 1.8 Hz) which was seen to be meta coupled to H-6' at δ 6.89 (J = 8.3, 1.8 Hz) was thus assigned to H-2'. Another pair of *ortho* coupled doublets at δ: 7.03 and 7.59 with coupling constant value 9.2 Hz were assigned to the two protons at position 7 and 8. The three hydrogen methoxy proton signal at δ 3.77 was seen to have HMBC correlation to C-3' implying the OMe group to be attached to C-3. The multiplet signals at δ: 3.43 and 3.71 were assigned to the two hydrogens attached to C-9'. Another multiplet at δ 4.33 which integrated for one proton and was coupled to the doublet signal at δ 5.12 was assigned to H-8'. Hence the remaining doublet signal at δ 5.12 was assigned to H-7'.

The ^{13}C NMR spectrum indicated a total of 23 carbons. The DEPT experiment showed the presence of 1 methyl, 1 methylene, 9 methine and 12 quarternary carbons. The twelve quarternary carbon signals which are δ: 12.9 (C-1), 165.5 (C-3), 157.2 (C-4a), 101.9 (C-9a), 179.2 (C-9), 145.6 (C-10a), 131.5 (C-8a), 114.2 (C-5), 149.0 (C-6), 126.6 (C-1'), 147.7 (C-3') and 147.3 (C-4') were successfully assigned (Table-1). Carbon signals at δ: 162.9 (C-1), 165.5 (C-3), 147.7 (C-3'), 147.3 (C-4') and 59.9 (C-9') were oxygenated carbons. The conjugated carbonyl signal at δ 179.2 was assigned to C-9. The HMQC spectrum allows the twelve protons to be assigned to their respective carbons.

The positions of the hydroxyls and methoxyl substituents were confirmed unambiguously through the HMBC spectrum (Fig. 1).

TABLE-1
^1H NMR (400 MHz, DMSO-d_6) AND ^{13}C NMR (100 MHz, DMSO-d_6) ASSIGNMENTS OF 5'-DEMETHOXYCADENSIN G

Position	δ_H	δ_C	^1H-^1H COSY	HMBC
1	–	162.9	–	–
2	6.20 (1H, d, J = 1.8 Hz)	98.3	–	162.9 (C-1) (^2J), 165.5 (C-3) (^2J), 94.1 (C-4) (^3J), 101.7 (C-9a) (^3J)
3	–	165.5	–	–
4	6.39 (1H, d, J = 1.8 Hz)	94.1	–	98.3 (C-2) (^3J), 165.5 (C-3) (^2J), 157.2 (C-4a) (^2J), 101.7 (C-9a) (^3J)
4a	–	157.2	–	–
5	–	114.2	–	–
5a	–	145.6	–	–
6	–	149.0	–	–
7	7.03 (1H, t, J = 9.2 Hz)	113.8	7.59 (H-8)	114.2 (C-5) (^3J), 131.5 (C-8a) (^3J)
8	7.59 (1H, d, J = 9.2 Hz)	116.7	7.03 (H-7)	149.0 (C-6) (^3J), 126.6 (C-5a) (^3J)
8a	–	131.5	–	–
9	–	179.2	–	–
9a	–	101.7	–	–
1'	–	126.6	–	–
2'	7.04 (1H, d, J = 1.8 Hz)	111.8	–	147.3 (C-4') (^3J), 120.7 (C-6') (^3J), 76.5 (C-7') (^3J)
3'	–	147.7	–	–
4'	–	147.3	–	–
5'	6.82 (1H, d, J = 8.3 Hz)	115.4	6.89 (H-6')	126.6 (C-1') (^3J), 147.7 (C-3') (^3J)
6'	6.89 (1H, dd, J = 8.3, 1.8 Hz)	120.7	6.82 (H-5')	111.8 (C-2') (^3J), 147.3 (C-4') (^3J)
7'	5.12 (1H, d, J = 7.3 Hz)	76.5	4.33 (H-8')	126.6 (C-1') (^2J), 78.0 (C-8') (^2J)
8'	4.33 (1H, m)	78.0	5.12 (H-7')	–
9'	3.43 (1Ha, m) 3.71 (1Hb, m)	59.9	–	–
1-OH	12.95 (OH, s)	–	–	162.9 (C-1) (^2J), 98.3 (C-2) (^3J), 101.7 (C-9a) (^3J)
3'-OCH_3	3.77 (3H, s)	55.7	–	147.7 (C-3') (^3J)

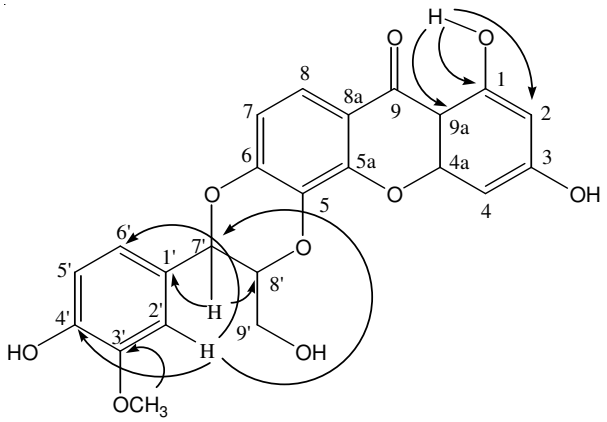


Fig. 1. Important HMBC correlations of 5'-demethoxycadensin G (**1**)

From the HMBC experiment, the chelated O-H at δ 12.95 gave linkages to carbons C-1, C-2 and C-9a, thus supporting its position at C-1. The proton signal at δ 7.04 was correlated to δ 147.3 (C-4'), 120.7 (C-6') and 76.5 (C-7') by ^3J and ^2J correlations. Meanwhile, H-7' (δ 5.12) correlated to δ 78.0 (C-8') (^2J) and δ 126.6 (C-1') (^2J) confirming its attachment to C-7'. Other HMBC correlations are shown in Fig. 1.

Therefore, compound **1** was elucidated to be 5'-demethoxycadensin G (**1**) previously isolated from *Cratoxylum cochinchinense*[6]. The complete spectral data for **1** are summarized in Table-1.

DPPH, a compound that possesses a free radical proton is readily destroyed by proton radical scavengers. Its characteristic absorption in the 510-520 nm UV-visible region decreases significantly upon exposure to proton radical scavengers[18]. This assay tests the abilities of the function of the extracts of these two *Cratoxylum* species as a proton radical scavenger or hydrogen donor. This is the first report on the free-radical scavenging properties for these two plant species. The EC_{50} value for the methanol extracts of *Cratoxylum glaucum* and *C. arborescens* were 7.48 and 10.40 ppm, respectively, indicating a very high free radical scavenging activity against DPPH radical which is comparable to that of ascorbic acid (EC_{50} = 5.17 ppm) Table-2.

The total phenolic content of methanol extracts (0.1 mg/mL) of *Cratoxylum glaucum* and *C. arborescens* were found to be equivalent to 1.33 and 1.45 mg/mL gallic acid, respectively with calibration equation for gallic acid y = 0.009x + 0.021 (R^2 = 0.997). These values are in correlation with the DPPH radical scavenging activity, indicating a high contribution of the phenolic constituents towards the strong antioxidant property of these two plant species. This shows that these two *Cratoxylum* species could be potential sources of natural food antioxidant.

TABLE-2
FREE RADICAL SCAVENGING ACTIVITY OF *Cratoxylum arborescens* AND *C. glaucum* AGAINST DPPH

Plant species	Extracts	Calibration equation		EC_{50} (ppm)
C. glaucum	Hexane	y = 0.1540x + 0.7862	R^2 = 0.9958	319.57
	Ethyl acetate	y = 0.4011x + 3.5714	R^2 = 0.9895	115.75
	Methanol	y = 6.5436x + 1.0256	R^2 = 0.9971	7.48
C. arborescens	Hexane	y = 0.1733x + 1.7735	R^2 = 0.9958	278.28
	Chloroform	y = 1.5479x + 1.9599	R^2 = 0.9924	31.04
	Methanol	y = 4.7461x + 0.6642	R^2 = 0.9981	10.40
Ascorbic acid		y = 10.132x - 2.4082	R^2 = 0.9939	5.17

ACKNOWLEDGEMENTS

Financial support from the Malaysian Science Fund Grant is acknowledged. The authors also wish to thank Assoc. Prof. Dr. Jegak Uli for collection of plant samples, Mr. Johadi Iskander for recording NMR spectra and Mr. Zainal Abidin Kassim for recording MS spectra.

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