Phenolic compounds from the flowers of *Garcinia dulcis*

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Abstract

Dulcisxanthones C–F (1–4) and dulcinone (5) together with 22 known compounds were isolated from the flowers of *Garcinia dulcis*. Their structures were determined by spectroscopic methods. The abilities of some of these compounds to act as radical scavengers and antibacterial agents were investigated.

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Keywords: *Garcinia dulcis*; Guttiferae; Xanthones; Chromones; Radical scavenging; Antibacterial

1. Introduction

The sub-woody plant *Garcinia dulcis* Kurz. (Guttiferae), local Thai name Ma-Phut, grows mainly in southeast Asia. Its leaves and seeds have been used in traditional medicine against lymphatitis, parotitis, struma and other disease conditions (Kasahara and Henmi, 1986). In our previous work (Deachathai et al., 2005), we have investigated the chemical constituents from its fruit and their biological activities. In our continuing work, we have examined the chemical constituents from the flowers. This investigation had led to the isolation and structural determination of five new and 22 known compounds.

2. Results and discussion

The flowers of *G. dulcis* were sequentially extracted with acetone. Purification of the extract, produced four new xanthones named dulcisxanthones C–F (1–4), one new chromone named dulcinone (5), along with 22 known compounds: volkensiflavone (6), morelloflavone (7) (Ansari et al., 1976), 1-hydroxy-3,4,5-trimethoxyxanthone (8) (Stout et al., 1973), rhamnazin (9) (Subhadhirasakul et al., 2003), quercetin 3-O-β-galactopyranoside (10) (Kartnig et al., 1985), podocarpusflavone A (11) (Harrison et al., 1994), xanthochymusside (12), fukugeside (13) (Konomi et al., 1970), cowaxanthone (14) (Deachathai et al., 2005), GB-2a (15) (Ansari et al., 1976), xanthochymol (16) (Blount and Williams, 1976), BR-xanthone A (17), 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)-xanthone (18) (Deachathai et al., 2005), guttiferone E (19) (Gustafson et al., 1992), rhedixanthone A (20) (Delle Monache et al., 1981), α-mangostin (21), 1,7-dihydroxy-3-methoxy-2-(3-methyl-2-butenyl)xanthone (22) (Deachathai et al., 2005), 3-isomangostin (23) (Mahabusarakam et al., 1987), kaempferol 3-O-glucopyranosyl-7-O-rhamnopyranoside (24), garcinone B (25), morusignin J (26) (Deachathai et al., 2005) and β-mangostin (27) (Mahabusarakam et al., 1987).

Dulcisxanthone C (1) is 1-hydroxy-2,3,4,6-tetramethoxyxanthone, and a yellow solid, m.p. 125–128°C. Its
molecular formula of C$_{17}$H$_{16}$O$_{7}$ was established on the basis of its mass spectrum ([M]$^+$ $m/z$ 332). The UV spectrum showed absorption maxima at 376, 309, 273, 241 nm. The IR spectrum exhibited O–H stretching at 3400 cm$^{-1}$ and C=O stretching at 1646 cm$^{-1}$. The $^1$H NMR spectrum showed a singlet signal of a chelated hydroxy proton (1-OH) at $\delta$ 12.60. The signals in the aromatic region, $\delta$ 7.84 ($dd$), 7.32 ($d$) and 7.26 ($d$) that appeared as an ABX type were proposed for the signals of H-7, H-8 and H-5, respectively. These assignment were supported by the correlations of H-7 to C-5, C-6, C-9; H-8 to C-6, C-8a, C-10a and H-5 to C-6, C-7, C-10a from an HMBC experiment. Four singlet signals at $\delta$ 4.04, 4.16, 3.97 and 4.05 were assigned for the methoxy protons of 2-OCH$_3$, 3-OCH$_3$, 4-OCH$_3$ and 6-OCH$_3$, respectively, and confirmed by the $^3$J coupling of the methoxy protons to C-2, C-3, C-4 and C-6, respectively, in HMBC. The assigned structure was further confirmed by analysis of HMBC correlations (Table 1).

Dulcisxanthone D (2) is 1,6-dihydroxy-5-(3-methyl-2-buteryl)-2′,2′ ′-dimethylchromeno(5′,6′;2,3)-2″,2″′-dimethylchromeno(5″,6″;8,7)xanthone. It is an orange solid, m.p. 218–220°C, with the molecular formula C$_{28}$H$_{28}$O$_{6}$. The UV spectrum showed absorption maxima at 335, 301, 289, 221 nm. Absorption bands of O–H stretching and C=O stretching were shown in the IR spectrum at 3424 cm$^{-1}$ and at 1615 cm$^{-1}$. The 1H NMR spectrum showed the signals of a chelated hydroxy proton at $\delta$ 13.70 (1-OH), a non-chelated hydroxy proton at $\delta$ 6.34 (6-OH) and an isolated aromatic proton at $\delta$ 6.30 (H-4). The characteristic signals of a prenyl group were present at $\delta$ 3.57 (H-1″), 5.28 (H-2″), 1.69 (H-4″) and 1.88 (H-5″) and it was located at C-5 according to the HMBC correlation of H-1″ to C-6, and C-10a. The signals of four methyl groups at $\delta$ 1.49 (6H, s, 2′-Me) and 1.47 (6H, s, 2″-Me) and vicinal olefinic protons at $\delta$ 5.77 (d, H-3″″), 7.98 (d, H-4″″), 5.57 (d, H-3′′) and 6.73 (d, H-4′′) associated with a chromene rings were present. The $^3$J correlations of H-4″″ to C-7 and H-4′′ to C-1 suggested that the chromene rings were connected to the parent structure at C-7, C-8 and C-2, C-3. The complete HMBC data (Table 2) confirmed this structure.

Dulcisxanthone E (3) is 1,3,6,7-tetrahydroxy-2-(3,7-dimethyl-2,6-octadienyl)-5-(3-methyl-2-buteryl)xanthone. It is a yellow solid with the molecular formula C$_{28}$H$_{32}$O$_{6}$. The UV spectrum showed absorption maxima at 369, 322, 258, 242 nm. The IR spectrum showed absorption bands of O–H stretching at 3450 cm$^{-1}$ and C=O stretching at 1690 cm$^{-1}$. The 1H NMR spectrum exhibited resonances of a chelated hydroxy proton 1-OH at $\delta$ 13.19 and aromatic protons H-4 at $\delta$ 6.42 and H-8 at $\delta$ 7.33. The characteristic
signals of a prenyl unit were displayed at $\delta$ 6.30 ($d$, H-1'), 5.28 ($br t$, H-2'), 1.69 ($s$, H-4') and 1.88 ($s$, H-5'). The prenyl unit was linked to C-5 according to the HMBC correlation of H-1' to C-5, C-6 and C-10a. It also exhibited the typical signal of a geranyl group: three singlets of three methyl groups at $\delta$ 1.57 (H-8'), 1.80 (H-9') and 1.52 (H-10'), a doublet of methylene protons at $\delta$ 3.37 (H-1'), two multiplets of methylene protons at $\delta$ 1.99 (H-4') and 2.03 (H-5') and two broad triplets of two olefinic methine protons at $\delta$ 5.28 (H-2') and 5.02 (H-6'). The correlation of H-1' to C-1, C-2 and C-3 in the HMBC indicated that the geranyl side chain was at C-2. The complete HMBC (Table 2) supported the assigned structure.

Dulcinone (5) is 6,8-dihydroxy-2,7-dimethyl-4H-chromen-4-one. It is a yellow solid with molecular formula C_{24}H_{24}O_{6}. The UV spectrum showed absorption maxima at 3343 cm$^{-1}$ and C=O stretching at 1657 cm$^{-1}$. The $^{13}$C NMR spectrum also showed the resonance of a carbonyl carbon at $\delta$ 181.5. The $^1$H NMR spectrum showed a singlet signal of an olefinic proton H-3 at $\delta$ 5.99, a singlet signal of an aromatic proton H-5 at $\delta$ 6.33, a singlet signal of methyl protons 2-Me at $\delta$ 2.39 and a singlet signal of methyl protons 7-Me at $\delta$ 2.13. These assignment were supported by the HMBC correlations: H-3 to C-2, C-4, C-4a; H-5 to C-4a, C-6, C-7, C-8a; 2-Me to C-2, C-3 and 7-Me to C-6, C-7, C-8. The assigned structure was further confirmed by HMBC correlations (Table 1).

Comounds 1–5 are new natural products. Compounds 6, 7, 11, 14, 15, 17, 18, 21, 22, 24, 25, and 26 were previously isolated from G. dulcis (Ansari et al., 1976; Harrison et al., 1994; Deachathai et al., 2005). This is the first report of the known compounds 8, 9, 10, 12, 13, 16, 19, 20, 23, 27 in G. dulcis. Evaluation of the radical scavenging activities of some of the compounds at the concentration of 10 $\mu$m (Table 3) revealed that none of the newly isolated compounds showed any antioxidant activity. However 10, 13, 16 and 19 had potent antioxidant properties produced 57%, 56%, 60% and 59% scavenging properties, respec-

### Table 2

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tively. These values corresponded to IC50 values of 10.50, 11.40, 8.50 and 10.10 μM, respectively. Compound 9 and 16 were tested for the antibacterial activity. Compound 16 was found to inhibit the growth of both the penicillin-sensitive strain, ATCC 25923, and the methicillin-resistant strain, MRSA SK1, of Staphylococcus aureus with MIC values of 8 μg/mL whereas 9 showed only weak activity with MIC values greater than 128 μg/mL. The results of radical scavenging and antibacterial activities of compounds 7, 14, 17, 18, 21, 22, 24, 25, 26 have been reported in the previous work (Deachathai et al., 2005).

3. Experimental

3.1. General methods

Melting points were measured on a digital Electrothermal 9100 Melting Point Apparatus and are uncorrected. Infrared spectra were recorded on an FTS 165 FT-IR spectrometer. Ultraviolet absorption spectra were recorded on a digital Electrothermal 5100 Melting Point Apparatus and are uncorrected. 

3.2. Plant material

The flowers of G. dulcis were collected from Songkhla province in the southern part of Thailand. The voucher specimen (Coll. No. 02, Herbarium No. 0012652) has been deposited at Prince of Songkla University Herbarium, Biology Department, Faculty of Science, Prince of Songkla University, Thailand.

3.3. Extraction and isolation

The flowers of G. dulcis (1.2 kg) were extracted at room temperature sequentially with acetone (5 and 7 days). Removal of the solvents from the extracts yielded the acetone extracts A (148.2 g) and B (38.4 g). An aliquot of the acetone extract A (92.0 g) was further separated by QCC and eluted with CH2Cl2, CH2Cl2–Me2CO, Me2CO, Me2CO–MeOH gradient solvent system. The eluted fractions were combined into 17 fractions (A1–A17) on the basis of their TLC behaviour. Fraction A6 (7.7 g) was subjected to CC on sephadex LH-20 and eluted with a gradient of H2O–MeOH to give compounds 1 (3.0 mg), 8 (9.0 mg), 9 (62.0 mg), 10 (27.0 mg) and 11 (10.0 mg). Fractions A5 (2.2 g), A7 (4.8 g) and A13 (1.0 g) were further purified producing 6 (29.1 mg), 7 (1.5 g), 12 (148.0 mg) and 13 (51.0 mg). The acetone extract A (55.8 g) was also fractionated by dissolving in CH2Cl2 to give a soluble (15.2 g) and insoluble (40.2 g) fraction. This CH2Cl2 soluble fraction was subjected to silica gel CC and eluted sequentially with CH2Cl2, and a CH2Cl2–Me2CO gradient to afford 2 (10.0 mg), 14 (21.5 mg), 15 (38.2 mg) and 16 (2.3 g). The CH2Cl2 insoluble fraction after CC produced 17 (15.2 g) and 18 (5.4 mg). Extraction of the acetone extract B (38.4 g) with CH2Cl2, EtOAc and then Me2CO gave CH2Cl2 soluble – (8.7 g), EtOAc soluble – (8.5 g) and Me2CO soluble (3.5 g) fractions. After CC of CH2Cl2 soluble fraction and eluting with CH2Cl2–Me2CO in a polarity gradient manner, compounds 19 (10.2 mg), 20 (5.5 mg), 3 (14.3 mg), 21 (31.7 mg), 22 (20.2 mg), 23 (3.2 mg) and 24 (12.3 mg) were obtained. The EtOAc soluble fraction was subjected to silica gel CC eluted with CH2Cl2–Me2CO in a polarity gradient manner to afford 4 (23.8 mg) and 5 (5.2 mg), whereas the Me2CO soluble fraction subjected to silica gel CC and eluted with CH2Cl2–MeOH in a polarity gradient manner gave 25 (2.6 mg), 26 (2.3 mg) and 27 (15.2 mg).

3.3.1. Dulcisxanthone C (1)

Yellow solid, m.p. 125–128 °C. HRESIMS m/z 332.0892 [M]+ (calcd. for C17H16O7, 332.0896). UV (CH3OH) \( \lambda_{max} \) (nm) (log e): 376 (3.37), 309 (3.78), 273 (3.87), 241 (3.91). IR (neat), \( \nu \) (cm\(^{-1}\)): 3400, 1646. 1H NMR (CDCl3) (δ ppm): 12.60 (1H, s, 1-OH), 7.84 (1H, dd, J = 7.8, 1.5 Hz, H-8), 7.32 (1H, d, J = 7.8 Hz, H-7), 7.26 (1H, d, J = 1.5 Hz, H-5), 4.16 (3H, s, 3-OHMe), 4.05 (3H, s, 6-OHMe), 3.63 (4H, s, 2-OHMe), 3.97 (3H, s, 4-OHMe). EIMS m/z (% rel. int.): (M–332) 332, 100, 317 (98), 302 (21), 287 (15), 259 (17), 203 (10), 175 (13). 13C NMR (CDCl3) (δ ppm): 181.7 (C-9), 154.1 (C-3), 150.6 (C-1), 148.7 (C-6), 146.4 (C-10a), 145.7 (C-4a), 135.4 (C-4), 132.7 (C-2), 123.7 (C-8), 120.9 (C-8a), 116.5 (C-7), 115.9 (C-5), 104.5
(C-9a), 61.9 (2-OMe), 61.7 (3-OMe), 61.2 (4-OMe), 56.5 (6-OMe).

3.3.2. Dulcisxanthone D (2)

Yellow solid, m.p. 218–220 °C. HRFABMS m/z 460.1929 [M]+ (calcd. for C28H28O6 460.1886). UV (CH3OH) λmax (nm) (log ε): 335 (4.10), 301 (4.24), 289 (4.30), 221 (4.21), 205 (4.25). IR (neat) ν (cm−1): 3424, 1615, 1440 cm−1. 1H NMR (CDCl3) (δ ppm): 13.70 (1H, s, 1-0H), 7.98 (1H, d, J = 10.2 Hz, H-4″), 6.73 (1H, d, J = 10.2 Hz, H-4′′), 6.34 (1H, s, 6-0H), 6.30 (1H, s, H-4), 5.77 (1H, d, J = 10.2 Hz, H-3″), 5.57 (1H, d, J = 10.2 Hz, H-3′′), 5.28 (1H, br t, J = 5.7 Hz, H-2″), 3.57 (2H, d, J = 7.5 Hz, H-1″), 1.88 (3H, s, H-5″), 1.69 (3H, s, H-4″), 1.49 (6H, s, 2″-Me), 1.47 (6H, s, 2′-Me). FABMS m/z (% rel. int.): ([M]+ 460, 100), 444 (50), 61 (42). 13C NMR (CDCl3) (δ ppm): 180.6 (C-9), 162.5 (C-3), 159.6 (C-1), 155.5 (C-4a), 153.0 (C-10a), 150.7 (C-6), 136.7 (C-7), 132.2 (C-3′′), 131.7 (C-3″), 122.2 (C-2″), 121.0 (C-4″), 119.7 (C-8), 111.4 (C-2), 108.7 (C-8a), 102.2 (C-9a), 88.9 (C-4), 71.0 (C-2′′), 55.8 (3-OCH3), 29.6 (2″-Me), 27.3 (2′″-Me), 25.8 (C-4″), 21.3 (C-1″), 17.7 (C-5″).

3.3.5. Dulciteone (5)

Yellow solid, m.p. 227–229 °C. HRESIMS m/z 206.0570 [M]+ (calcd. for C12H15O4, 206.0579). UV (CH3OH) λmax (nm) (log ε): 327 (3.51), 300 (3.62), 258 (4.25), 226 (3.96). IR (neat) ν (cm−1): 3343, 1657, 1598, 1417. 1H NMR (CDCl3 + DMSO-d6 one drop) (δ ppm): 6.33 (1H, s, H-5), 5.99 (1H, s, H-3), 2.39 (3H, s, H-2), 2.13 (3H, s, H-7). EIMS m/z (% rel. int.): ([M]+ 206, 100), 205 (95), 177 (11), 165 (8). 13C NMR (CDCl3 + DMSO-d6 one drop) (δ ppm): 181.5 (C-4), 165.6 (C-2), 160.9 (C-6), 158.2 (C-8a), 154.6 (C-8), 106.8 (C-3), 102.9 (C-4a), 101.2 (C-7), 97.5 (C-5), 19.3 (2-Ch3), 6.4 (7-Ch3).

3.4. Radical scavenging activity

This was carried out according to the procedure of Deachatthai et al. (2005).

3.5. Antibacterial activity

This was carried out according to the procedure of Mahabusarakam et al. (2004).

Acknowledgements

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References


