Antioxidant Xanthones from the Pericarp of *Garcinia mangostana* (Mangosteen)

HYUN-AH JUNG,†,‡ BAO-NING SU,† WILLIAM J. KELLER,‡ RAJENDRA G. MEHTA,§ AND A. DOUGLAS KINGHORN*,†

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210; Nature’s Sunshine Products, Inc., 1655 North Main Street, Spanish Fork, Utah 84660; and Carcinogenesis and Chemoprevention Division, Illinois Institute of Technology Research Institute, Chicago, Illinois 60616

As part of ongoing research on cancer chemopreventive agents from botanical dietary supplements, *Garcinia mangostana* L. (commonly known as mangosteen) was selected for detailed study. Repeated chromatography of a CH₂Cl₂-soluble extract of the pericarp led to the isolation of two new highly oxygenated prenylated xanthones, 8-hydroxy cudraxanthone G (1) and mangostingone [7-methoxy-2-(3-methyl-2-buteryl)-8-(3-methyl-2-oxo-3-butenyl)-1,3,6-trihydroxyxanthone, 2], together with 12 known xanthones, cudraxanthone G (3), 8-deoxygartanin (4), garcinamgosone B (5), gincone D (6), gincone E (7), gartanin (8), 1-isomangostin (9), α-mangostin (10), γ-mangostin (11), mangostinone (12), smeatxanthone A (13), and tovophyllin A (14). The structures of compounds 1 and 2 were elucidated by spectroscopic data analysis. Except for compound 2, which was isolated as a minor component, the antioxidant activities of all isolates were determined using authentic and morpholinosydnonimine-derived peroxynitrite methods, and compounds 1, 8, 10, 11, and 13 were the most active. α-Mangostin (10) inhibited 7,12-dimethylbenz[a]anthracene-induced preneoplastic lesions in a mouse mammary organ culture assay with an IC₅₀ of 1.0 µg/mL (2.44 µM).

**KEYWORDS:** *Garcinia mangostana*; mangosteen; Clusiaceae; prenylated xanthones; antioxidant activity; mouse mammary organ culture assay

**INTRODUCTION**

It is well-recognized that consumption of fruits and vegetables can reduce the incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction, and cataracts (1–3). These protective effects are considered mainly to be due to the presence of various antioxidants in fruits and vegetables. Numerous investigations have indicated that free radicals cause oxidative damage to lipids, proteins, and nucleic acids (4, 5). Antioxidants seem to be very important in the prevention of these diseases, because they can inhibit or delay the formation of oxidizable substrate chain reactions (6–8).

*Garcinia mangostana* L. (Clusiaceae), commonly known as mangosteen, is a slow-growing tropical evergreen tree with leathery, glabrous leaves. The tree can attain 6–25 m in height and is mainly found in India, Myanmar, Sri Lanka, and Thailand. Mangosteen has dark purple to red-purple fruits. The edible fruit aril is white, soft, and juicy with a sweet, slightly acid taste and a pleasant aroma (9). The pericarp of mangosteen has been used in Thai indigenous medicine for the treatment of skin infections, wounds, and diarrhea for many years (9–11). Recently, products manufactured from *G. mangostana* have begun to be used as a botanical dietary supplement in the United States, because of their potent antioxidant potential (12). The major secondary metabolites of mangosteen have been found to be prenylated xanthone derivatives (10, 13–15); some members of this compound class isolated from this plant possess antifungal (16), antimicrobial (14), antioxidant (17), and cytotoxic (18) activities.

In the course of our ongoing research project on the chemical constituents and biological activity evaluation of popular herbal remedies (19), a CH₂Cl₂-soluble partition of the MeOH extract of the pericarp of mangosteen was found to have significant antioxidant activity in a peroxynitrite-scaevenging bioassay. This extract was then purified by repeated chromatography, which led to the isolation of two new highly oxygenated prenylated xanthones, 8-hydroxy cudraxanthone G (1) and mangostingone (2), as well as 12 known xanthones. The structure elucidation of compounds 1 and 2, the antioxidant activity evaluation of all isolates obtained in this investigation except compound 2, and the evaluation of α-mangostin (10) and γ-mangostin (11)
in a mouse mammary organ culture ex vivo assay were carried out.

MATERIALS AND METHODS

General Experimental Procedures. Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. The UV spectra were obtained with a Beckman DU-7 spectrophotometer, and the IR spectra were run on an ATI Mattson Genesis FTIR spectrometer. NMR spectrometric data were recorded at room temperature on a Bruker Advance DPX-300 or a DRX-400 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Standard pulse sequences were employed for the measurement of 2D NMR spectra (1H–1H COSY, HMBC, HMBC, and NOESY). Electrospray ionization (ESI) mass spectrometric analysis was performed with a 3-T Finnigan FTMS-2000 Fourier transform mass spectrometer.

5-Hydroxy-2-methoxy-4-methyl-3-phenyl-1H-pyrazole was isolated as a major component from the freeze-dried powder of the pericarp of G. mangostana (1 kg) was extracted by maceration with MeOH (3 L) at room temperature, for 3 days each. After filtration and evaporation of the CH2Cl2-soluble extract was subjected to chromatography over a Sephadex LH-20 column using MeOH as eluent, yielding seven subfractions (F1701–F1707). Fraction F1702 (200 mg) was chromatographed over a silica gel column with n-hexane/EtOAc (4:1) as solvent system to afford 1-isomangostin (9, 35 mg) and garcinomangoside B (5, 3 mg), in order of polarity. F1707 was separated using a semi-preparative reversed-phase HPLC column with H2O/CH3CN (15:85) at a flow rate of 7.0 mL/min to give mangostone (12; 6 mg; tR = 28.0 min) and smeathxanthone A (13; 8 mg; tR = 45.0 min). F1706 was purified with a Sephadex LH-20 column using pure MeOH as solvent, to give γ-mangostin (11, 600 mg). Fraction F18 was fractionated over a silica gel column with CHCl3/EtOAc (40:1) as eluent, resulting in 12 subfractions (F1801–F1812). The major subfraction, F1805 (6 g), was chromatographed over a Sephadex LH-20 column, eluting with pure MeOH, to afford another major isolate, γ-mangostin (11; 2 g), and seven subfractions (F180501–F180507). F180502 (100 mg) was purified over a silica gel column with CHCl3/acetone (35:1) as solvent, to afford an additional amount of 1-isomangostin (9, 20 mg). F180504 (90 mg) was chromatographed over a reversed-phase silica gel column eluted with MeOH/H2O (7:3), to yield garcinone D (6; 10 mg).

8-Hydroxyudranxanthone G (1) was obtained as a yellow solid: UV (MeOH) λmax (log e) 234 (2.88), 263 (4.38), 279 (4.34), 351 (3.97) nm; IR (dried film) νmax 3365, 1608, 1578, 1465, 1284, 1162, 1081 cm−1; 1H NMR (300 MHz, CDCl3) δ 12.16 (OH), 11.22 (OH), 7.25 (1H, d, J = 9.0 Hz, H-6); 6.69 (1H, d, J = 9.0 Hz, H-7); 5.24 (2H, m, H-2’ and H-3’); 3.81 (3H, s, OCH3-3), 3.54 (2H, d, J = 6.2 Hz, H-1’); 3.41 (2H, d, J = 6.9 Hz, H-1’); 1.87 (3H, s, H-5’), 1.81 (3H, s, H-5’), 1.74 (3H, s, H-4’); 13C NMR (75 MHz, CDCl3) δ 185.4 (C-9), 164.4 (C-3), 158.3 (C-1), 152.7 (C-4a), 142.8 (C-10a), 135.9 (C-3), 132.3 (C-3’), 132.2 (C-3’), 123.0 (C-6), 121.0 (C-2’), 122.2 (C-2’), 118.2 (C-2), 113.0 (C-4), 109.8 (C-7), 107.3 (C-9a), 104.9 (C-1a), 62.1 (OCH3-3), 25.7 (C-4’), 25.5 (C-4’), 23.0 (C-1’), 22.5 (C-1’), 18.0 and 17.9 (C-5’ and C-5’); HRESIMS m/z 433.1614 [M + Na]+ (calcd for C20H13O10Na+, 433.1621).

Mangostone (2) was obtained as a yellow solid: UV (MeOH) λmax (log ε) 243 (3.84), 320 (3.65), 354 (3.32) nm; IR (dried film) νmax 3365, 1608, 1578, 1465, 1284, 1162, 1081 cm−1; 1H NMR (300 MHz, acetone-d6) δ 13.50 (OH), 6.86 (1H, s, H-5), 6.39 (1H, H-4), 6.23 (1H, s, H-4’a), 5.86 (1H, s, H-4’b), 5.24 (1H, t, J = 6.8 Hz, H-2’), 4.75 (2H, s, H-1’), 3.73 (2H, s, OCH3-3), 3.30 (2H, d, J = 6.8 Hz, H-1’), 1.92 (3H, s, H-5’), 1.75 (3H, s, H-4’), 1.61 (3H, s, H-5’); 13C NMR (75 MHz, acetone-d6) δ 191.1 (C-2’), 182.2 (C-9), 163.3 (C-3), 161.4 (C-1), 161.6 (C-1a), 156.1 (C-4a), 155.8 (C-10a), 145.8 (C-5’), 145.7 (C-7), 131.4 (C-3), 131.2 (C-3), 123.6 (C-4’), 111.0 (C-9a), 111.0 (C-2), 103.3 (C-5), 103.2 (C-1a), 93.4 (C-4’), 61.3 (OCH3-3), 37.9 (C-1’), 25.9 (C-4’), 22.0 (C-1’), 18.1 (C-5’ and C-5’); HRESIMS m/z 447.1432 [M + Na]+ (calcd for C20H12O11Na+, 447.1412).

Measurement of Peroxynitrite Scavenging Activity. ONOO−-scavenging activity was measured by monitoring the oxidation of nonfluorescent DHR 123 to highly fluorescent rhodamine 123 using the modified method of Kooy et al. (20). Briefly, DHR 123 (5 mM) in EtOH, purged with nitrogen, was stored at −80 °C as a stock solution. This solution was not exposed to light, prior to the study. The rhodamine buffer (pH 7.4) consisted of 50 mM sodium phosphate dibasic, 50 mM sodium phosphate monobasic, 90 mM sodium chloride, 5 mM potassium chloride, and 100 μM DTPA. The final concentration of DHR 123 was 5 μM. The buffer in this assay was prepared before use and placed on ice. The concentrations of compounds tested were in the range from 0.02 to 100 μg in 10% DMSO. The background and final fluorescence intensities were measured 5 min after treatment with and without the addition of authentic ONOO− in 0.3 N sodium hydroxide (10 μM) or SIN-1 in deionized water (10 μM). DHR 123 was oxidized rapidly by ONOO−, superoxide anion (O2•−), and nitric oxide (NO•). The fluorescence intensity of oxidized DHR 123 was measured with an LS55 luminescence spectrometer (Perkin-Elmer, Boston, MA) at the excitation and emission wavelengths of 480 and 530 nm, respectively. Values of ONOO− scavenging activity (50% inhibition, IC50) were expressed as the mean (n = 3) for the final fluorescence intensity
minus background fluorescence by the detection of oxidation of DHR 123. DL-Penicillamine was used as a positive control.

**Mouse Mammary Organ Culture Assay.** This assay was carried out according to an established protocol (21). In brief, 4-week-old BALB/c female mice (Charles River, Wilmington, MA) were pretreated for 9 days with 1 ìg of estradiol and 1 mg of progesterone. On the 10th day, the mice were sacrificed and the second pair of thoracic mammary glands was dissected on silk and transferred to 60 mm culture dishes containing 5 mL of Waymouth’s 752/1 MB medium supplemented with streptomycin, penicillin, and L-glutamine. The glands were incubated for 10 days (37 °C, 95% O₂ and 5% CO₂) in the presence of growth-promoting hormones (5 ìg of insulin, 5 ìg of prolactin, 1 ìg of aldosterone, and 1 ìg of hydrocortisone per milliliter of medium). Glands were exposed to 2 ìg/mL 7,12-dimethylbenz[a]anthracene (DMBA) between 72 and 96 h. After their exposure, glands were rinsed and transferred to new dishes with fresh medium. The fully differentiated glands were then permitted to regress by withdrawing all hormones except insulin for 14 additional days. Test compounds were present in the medium during days 1-10 of culture; mammary glands were scored for the incidence of lesions.

**RESULTS AND DISCUSSION**

Repeated column chromatography of the CH₂Cl₂-soluble fraction of the pericarp of *G. mangostana* led to the isolation of two new (1 and 2) and 12 known prenylated xanthones (Figure 1). The structures of the known compounds cudraxonanthone G (3) (22), 8-deoxygartanin (4) (23), garcimangosone B (5) (24), garcinone D (6) (25), garcinone E (7) (26), gartanin (8) (27), 1-isomangostin (9) (10), α-mangostin (10) (28), γ-mangostin (11) (26), mangostinone (12) (29), smeathxanthone A (13) (30), and tovophyllin A (14) (31) were identified by comparing their physical and spectroscopic data (UV, 1H NMR, 13C NMR, DEPT, and 2D NMR) with those of published values and were confirmed by their HRESIMS data. Compounds 10 and 11 were found to be the major components of the CH₂Cl₂-soluble extract of the pericarp of *G. mangostana*.

A molecular formula of C₂₄H₂₆O₆ was determined for compound 1 by its HRESIMS (m/z 433.16114 [M + Na]⁺). The 1H NMR spectrum revealed two downfield singlets at δH 11.22 and 12.16, suggesting the presence of two hydrogen-bonded hydroxy groups in the molecule of 1. The 1H NMR spectrum of this compound also displayed the characteristic signals of two ortho-coupled aromatic protons at δH 7.25 (1H, d, J = 9.0 Hz, H-6) and 6.69 (1H, d, J = 9.0 Hz, H-7), two olefinic protons at δH 5.24 (2H, m, H-2′ and H-2′′), one methoxy group at δH 3.81 (3H, OMe-3), and four tertiary methyls at δH 1.87 (3H, s, H-5′′), 1.81 (3H, s, H-5′), 1.74 (3H, s, H-4′′), and 1.71 (3H, s, H-4′). The 13C NMR spectrum of compound 1 showed 24 resonance signals. The presence of two 3-methylbut2-enyl functionalities in compound 1 could be assigned by interpretation of its 1H and 13C NMR spectroscopic data as well as the correlations observed in the 1H—1H COSY, HMBC, and HMOC spectra. In addition to the signals of these two prenyl groups and the signal of a typical methoxy substituent group, only 11 carbon resonance signals composed of two aromatic rings and one doubly conjugated carbonyl carbon (δC 185.4)
remained for compound 1. These NMR data suggested that compound 1 is a prenylated xanthone derivative (27, 29). The two downfield hydrogen-bonded hydroxy singlets at δH 14.1 and 12.16 suggested the locations of two of the three hydroxy groups to be at C-1 and C-8 in the molecule of 1. In the HMBC spectrum (Figure 2A), correlations were observed from H-6 to C-5, C-8, and C-10a, from H-7 to C-5, C-6, C-8, and C-9a, from OMe-3 to C-3, and from both H-1′ and H-1′′ to C-3. These correlations were used to assign the positions of two prenyl units and the methoxy group. Therefore, compound 1 was determined to be 8-hydroxycoumaroxanthone G.

A sodiated molecular ion peak at m/z 447.14323 [M + Na]+ in its HRESIMS was used to assign a molecular formula of C24 H24 O7 for compound 2. The UV (λmax at 243, 320, and 354 nm) and IR (λmax at 3365 (O=H), 1608 (C=O), and 1578 (aromatic ring) cm−1) spectroscopic data of compound 2 were very similar to those of 1. The 1H and 13C NMR spectroscopic data suggested that compound 2 is also a prenylated xanthone. In the 1H NMR spectrum of 2, only one downfield singlet for a hydrogen-bonded hydroxy group was displayed at δH 13.50 (OH-1). In addition to a methoxy group and the signals of the xanthone skeleton, ten other resonances were shown in the 13C NMR spectrum of 2. By interpretation of the chemical shifts and splitting patterns as well as the observed 2D NMR (1H COSY, HMQC, and HMBC) correlations of the nonskeletal protons and carbons, the two prenyl units in the molecule of 2 were determined to be 3-methylbut-2-ethyl and 2-oxo-3-methylbut-3-enyl, respectively. On the basis of the above-mentioned NMR data analysis and the determined molecular formula, the presence of three hydroxyl groups could be deduced. The positions of all substituents, namely, one methoxy group, two prenyl units, and three hydroxy groups, were assigned by careful analysis of the correlations obtained in the HMBC spectrum (Figure 2B). The observed key HMBC correlations for the structure assignment were from OH-1 to C-1a, C-1, and C-2, from H-1′ to C-1, C-2, and C-3, from H-1′′ to C-7, C-8, and C-9a, and from the methoxy singlet at δH 3.73 to C-7. Hence, compound 2, mangostingone, was determined to be 1,3,6-trihydroxy-7-methoxy-2-(3-methylbut-2-enyl)-8-(2-oxo-3-methylbut-3-enyl)-xanthone.

The antioxidant activities of 13 isolated compounds (1 and 3–14) were determined using the authentic ONOO• scavenging activity and SIN-1-derived ONOO• scavenging methods (20, 32). Compound 2 was obtained in insufficient amounts for this testing. The scavenging activities on ONOO• of the compounds tested are summarized in Table 1. Five of the xanthones (1, 8, 10, 11, and 13) were demonstrated to possess potent antioxidant activity in both assays tested. The species ONOO•, generated from NO• and O2•− in vivo, has been reported to act as an oxidant and be involved in the initiation of carcinogenesis, along with NO• (33). Because there is a lack of defense systems against ONOO• in the body and the highly reactive peroxynitrous acid (ONOOH), formed by protonation of ONOO−, easily decomposes to induce more highly reactive oxygen species, such as ‘OH, there is considerable interest in the development of ONOO• scavengers (34, 35). Until now, two possible pathways of phenolic compounds to scavenge ONOO• may be represented by nitration and electron donation. Monohydroxylated phenolic compounds, such as ferulic acid and p-coumaric acid, act as ONOO• scavengers by nitration. On the other hand, compounds with a catechol moiety, such as caffeic acid and chlorogenic acid, reduce ONOO• generated from NO• and O2•− by electron donation (36). The presence of two hydroxyl groups at the C-5 and C-8 positions in compounds 1, 8, and 13 was consistent with their potent antioxidant effects (37, 38). Compounds 10 and 11 both possess hydroxyl groups at positions C-1, C-3, and C-6.

The above results are supportive of the use of the pericarp of G. mangostana as an antioxidant botanical dietary supplement. It is worth noting that two of the active isolates obtained in the present investigation, α-mangostin (10) and γ-mangostin (11), were found to be major components of the CH2Cl2-soluble extract of the pericarp of G. mangostana. Therefore, these two compounds may be used as marker components for quality control of this botanical dietary supplement.

In addition to their peroxynitrite antioxidant activity, α-mangostin (10) and γ-mangostin (11) were evaluated for their potential to inhibit DMBA-induced preneoplastic lesions in a mouse mammary organ culture (MMOC) assay (39, 40). At a concentration of 10 μg/mL, the percent inhibitions of compounds 10 and 11 were 57.1% and 42.9%, respectively. The more active compound, α-mangostin (10), was then further evaluated in a dose–response MMOC assay, and it exhibited an IC50 of 1.0 μg/mL (2.44 μM) (Figure 3). As noted previously (21), substances active in this model system are considered to be good candidates for further investigation in full-term cancer chemo-
preventive studies in experimental animal models. In recent work, a crude α-mangostin (10) preparation was found to have efficacy in inhibiting preneoplastic lesions in a rat colon carcinogenesis model (41). Accordingly, the further investigation of extracts of mangosteen pericarp and α-mangostin as potential cancer chemopreventive agents seems to be warranted.

α-Mangostin (10) has been reported as a significant antimycobacterial substance against Mycobacterium tuberculosis with a minimum inhibitory concentration value of 6.25 μg/mL (12). In addition, this xanthone was found to be a histamine H1 receptor antagonist (42). α-Mangostin (10) was also reported as a selective inhibitor against bovine brain-derived acidic sphingomyelinase (43). Recently, synthetic methods for this prenylated xanthone have been reported (44, 45).

ACKNOWLEDGMENT

We are grateful to M. E. Hawthorne, Carcinogenesis and Chemoprevention Division, Illinois Institute of Technology Research Institute, Chicago, IL, for the technical assistance for MMOC testing of selected compounds. We thank J. Fowble, College of Pharmacy, The Ohio State University (OSU), for facilitating the running of the 300 and 400 MHz NMR spectra, and Dr. C. M. Hadad and S. Hatcher, Department of Chemistry, OSU, for the MS data.

LITERATURE CITED

(30) Bennett, G. J.; Harrison, L. J.; Sia, G.-L.; Sim, K.-Y. Triterpenoids, tocotrienols and xanthones from the bark of Cratoxylum cochinchinense. Phytochemistry 1993, 32, 1245–1251.

Received for review October 25, 2005. Revised manuscript received January 12, 2006. Accepted January 13, 2006. Faculty start-up funding from the cancer chemoprevention program of The Ohio State University Comprehensive Cancer Center to A.D.K. is gratefully acknowledged.