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Inhibition of Lipoprotein Oxidation by Prenylated Xanthones Derived from Mangostin

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Oxidative damage is thought to play a critical role in cardiovascular and other chronic diseases. This has led to considerable interest in the antioxidant activity of dietary compounds. Flavonoids have received the most attention and much is known about the structural requirements for antioxidant activity. However, little is known about the antioxidant activity of other plant derived phenolic compounds such as the xanthones. We have previously shown that the prenylated xanthone, mangostin, can inhibit the oxidation of low density lipoprotein. In order to examine the effects of structure modification on antioxidant activity of this class of compound we have prepared a number of derivatives of mangostin and tested antioxidant activity in an isolated LDL and plasma assay. The results of this study show that structural modification of mangostin can have a profound effect on antioxidant activity. Derivatisation of the C-3 and C-6 hydroxyl groups with either methyl, acetate, propane diol or nitrile substantially reduces antioxidant activity. In contrast, derivatisation of C-3 and C-6 with aminoethyl derivatives enhanced antioxidant activity, which may be related to changes in solubility. Cyclisation of the prenyl chains had little influence on antioxidant activity.

INTRODUCTION

There is good evidence to suggest that oxidative damage to lipoproteins, in particular low density lipoprotein (LDL), plays a critical role in the development of cardiovascular disease[1]. Oxidative modification to LDL produces particles containing lipid oxidation products and damaged protein which have enhanced atherogenic effects. Oxidised lipoproteins contribute to all stages of the atherosclerotic process, including activation of inflammatory events, endothelial damage, recruitment of macrophages and unregulated uptake of oxidised lipid particles by these cells to form foam cells, the hallmark of early atherosclerotic lesions[2]. Since oxidative damage plays a key role in cardiovascular and other degenerative diseases, there has been considerable interest in the role of antioxidants to inhibit these processes[3]. While endogenous lipophilic antioxidants such as α-tocopherol (vitamin E) no doubt play a role in protecting cell membranes and lipoproteins from oxidation we are particularly interested in plant derived antioxidant phenolic compounds which may be consumed in the diet[4]. In this regard the flavonoids have received major attention with a number of studies resolving the structural requirements for

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antioxidant activity[4,5,6,7]. In contrast relatively little is known about the antioxidant activity of the xanthones.

We have recently shown that a prenylated xanthone, mangostin (structure 1), isolated from the tropical fruit mangosteen, *Garcinia mangostana*, can inhibit LDL oxidation[8]. Mangostin appears to be the major bioactive compound derived from this tree and preparations from the fruit hull are used in traditional medicine in South East Asia[9,10]. This compound is a potent inhibitor of protein kinases and an antagonist of serotonin and histamine receptors[11,12]. In order to further explore the biological activity of this interesting compound, we have prepared a number of derivatives to observe effects on antioxidant activity and obtain information on structure/activity relationships for this class of compound.

METHODS

LDL purification

Blood was collected by venipuncture into EDTA (1g/L) and centrifuged immediately at 1000xg for 10min at 4°C. LDL was isolated by density gradient ultracentrifugation as previously described[13]. Briefly, plasma density was increased to 1.07 by addition of NaCl. A 4 step gradient was then constructed over the plasma using the following densities (kg/L): 0.5mL of 1.063 NaCl, 0.5mL of 1.04 NaCl, 0.5mL 1.02 NaCl and 0.9mL of double distilled water. To protect the LDL against oxidative modification during ultracentrifugation each density solution contained 100mg/L NaEDTA. Samples were ultracentrifuged at 205,000xg (average) for 20 hours at 4°C using a L-80 Ultracentrifuge (Beckman, USA). The LDL band was collected by aspiration and passed through a Pharmacia PD10 Sephadex column to remove the excess salt and the majority of the EDTA. The isolated LDL was stored in the dark at 4°C.

LDL oxidation studies

The LDL oxidation procedure was similar to that previously described by us[14]. Briefly, freshly isolated LDL was passed through a second Pharmacia PD10 Sephadex column to remove any remaining EDTA. The cholesterol concentration of the LDL was measured using a standard enzymatic method (Monotest, Boehringer Mannheim, Germany) and the protein content measured using the Lowry procedure with bovine serum albumin (Sigma) as the standard. The LDL was diluted with 0.15 M phosphate buffered saline (PBS), pre-treated with chelex chelating resin (Sigma), to a standard concentration of 0.1 mg/ml. Oxidation reactions were initiated by the addition of freshly prepared $CuSO_4$ (final concentration 5μmol/L) or 2,2'-azobis-(2-amidinopropane)hydrochloride (AAPH, final concentration 4mM) which breaks down thermally to produce peroxyl radicals.

For initial screening studies test compounds were dissolved in ethanol and added to the LDL to give a final concentration of 10μM. The total volume of ethanol in the buffer solution was 0.2%, and the same volume of ethanol was added to control oxidations. The extent of lipoprotein oxidation was determined by measuring the formation of lipid hydroperoxides at time points up to 90 minutes. Lipid hydroperoxides were measured by the ferrous oxidation-xylenol orange (FOX) assay[15] as previously described[16]. Antioxidant activity is expressed as the % inhibition of lipid hydroperoxide formation compared to control oxidations at 90 min. For dose response and time course studies, oxidation kinetics were determined by monitoring lipid hydroperoxides every 15 min over a 120 min period. For selected compounds doses ranging from 1 to 50 μM were used.

Serum oxidation ex vivo

Serum was prepared from blood left to clot in the dark at room temperature for 30min, then

centrifuged immediately at 1000xg for 10min at 4°C. Cu^{2+} (12.5μmol/L) induced serum oxidation (0.67% serum diluted in PBS) was carried out according to the method of Regnstrom et al[17] as previously described by us[16]. Oxidation kinetics were determined by measuring the absorbance at 234nm every 5min at 37°C for 330 min or until no further increase in the formation of conjugated dienes was observed.

α-Tocopherol analysis

LDL was mixed with an equal volume of cold methanol, and the mixture extracted with hexane. The hexane phase was dried under nitrogen and reconstituted in ethanol and immediately analysed by reverse-phase HPLC using electrochemical detection as previously described[14]. The mobile phase was methanol: ethanol (50:50 v\v) containing 2.5 g/L sodium perchlorate with a flow rate of 1 ml/min on a 25 cm Nucleosil C18 column (Alltech).

Experimental methods for preparation of mangostin derivatives

Melting points were measured by John melting point apparatus and are uncorrected. Infra-red spectra were recorded on a Beckman Acculab 3 Infrared Spectrophotometer and were recorded as potassium bromide disks unless otherwise stated. Ultra violet spectra were determined on a Beckman UV / VIS Spectrophotometer (model 126) in ethanol unless otherwise stated. Proton magnetic resonance spectra and irradiation experiments using $CDCl_3$ as solvent, unless otherwise stated, were obtained from a Bruker AC-200 (200 MHz) spectrometer with tetramethylsilane as internal reference. The chemical shifts were recorded in terms of ppm. Mass spectra were determined with an AEIMS-902 spectrometer (at 70 eV). Pre-coated silica gel 60 GF254 (Merck) was used for thin layer chromatography. Merck silica gel 70–250 mesh was used for column chromatography.

Extraction and Isolation of Mangostin (1)

The powdered, dried pericarps of *G. mangostana* (4 kg) were immersed in dichloromethane (20 l) for two days. The extraction was repeated twice, and the combined solution was concentrated (2.5 l). The yellow precipitate, which formed on cooling the solution, was collected (140 g). The solid was redissolved in dichloromethane and partitioned with 20% sodium carbonate. The organic layer was separated and washed with water several times until the water layer was no longer basic. The dried organic layer was concentrated and mangostin was recovered as a yellow crystalline solid (80 g), m.p. 180–182° (lit 182–183°)[18]. ^{1}H n.m.r. δ 1.70, 1.78, 1.84, 3 × s, 4 × CH3; 3.45, 4.11, d, J 7 Hz, C2 and C8-CH_2CH=; 5.28, bt, C2 and C8-CH_2CH=; 3.81, s, OCH_3 6.28, 6.82, 2 × s, H4 and H5; 13.80, s, C1 –0H; 6.31, s, ArOH. Mass spectrum m/z 410(61%), 367(30), 355(29), 354(28), 339(100), 323(18), 311(18), 311(18), 43(30). UV λmax (log ε) 243(4.54), 259(4.43), 318(4.35), 352(3.97). IR υmax (cm^{-1}) 3420(b), 3240, 1640, 1625, 1610, 1580.

Preparation of methyl derivative (2)

Mangostin (5 g) was methylated with methyl iodide (2 ml) and potassium carbonate in acetone overnight. The reaction products were chromatographed on a silica gel column. Elution with petroleum ether/dichloromethane (1:1) gave the methylated product (**2**) (1.5 g, 28%). **1-Hydroxy-3,6,7-trimethoxy-2,8-bis(3-methyl-but-2-enyl)-9*H*-xanthen-9-one (2)** m.p. 120–122° (lit × 123.3–123.80). ^{1}H n.m.r. δ 1.68, 1.80, 1.87, 3 × s, 4 × CH_3; 3.35, 4.13, d, J 7 Hz, C2 and C8-CH_2CH=; 5.24, 5.24, 2 × bt, C2 and C8-CH_2CH=; 3.80, s, OCH_3; 6.34, 6.75, 2 × s, H4 and H5; 13.63, s, C1 -OH; substituent group: 2 × OCH_3, 3.90, 3.95, 2 × s. Mass spectrum *m/z*. 438(70%), 395(60), 383(54), 367(100), 351(30), 339(30), 335(34). UV λ_{max} (log ε) 245(4.49), 262(4.52), 313(4.36), 348(3.83). IR ν_{max}(cm^{-1}) 3386(b), 1656, 1640, 1597, 1278, 1225, 1116.

Preparation of acetyl derivative (3)

Mangostin (1 g) was acetylated with acetic anhydride (2 ml) in pyridine (5 ml) at room temperature overnight. The crude product was chromatographed on silica gel. Elution with petroleum ether/dichloromethane (1:4) and recrystallization in methanol gave yellow needles of diacetylated product, (500 mg, 41 %) and monoacetylated product (3), (280 mg, 25%). **1,3-Dihydroxy-6-acetoxy-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (3)** m.p. 178–180°. ^{1}H n.m.r. δ 1.68, 1.77, 1.82, 3 × s, 4 × CH_3; 3.30, 4.07, 2 × d, C2 and C8-CH_2CH=, J 7 Hz; 5.15, 5.21, 2 × bt, C2 and C8-CH_2CH=., 3.80, s, OCH_3; 6.59, 6.80, 2 × s, H4 and H5; 13.63, s, C1 -OH; substituent group: 0Ac, 2.34, s. Mass spectrum *m/z* 452(68%), 409(18), 396(23), 381(15), 339(100), 323(21), 311(13), 43(62). UV λmax (log ε) 238(4.50), 250(4.44), 308(4.19), 362(4.00). IR ν_{max} (cm^{-1}) 3330(b), 1730, 1640, 1590, 1462, 1428, 1308, 1272.

Preparation of propanediol derivatives (4,5)

A solution of mangostin (1) (10 g, 24 mmol) in methanol (10 ml) was added to a solution of sodium (0.6 g) in methanol (4 ml). The mixture was stirred for 15 minutes then 3-chloro-1,2-propanediol (2.7 g, 25 mmol) and sodium iodide (3.7 g, 25 mmol) were added. The reaction mixture was refluxed for 20 hours then poured into ice water, neutralised with 3M HCl and extracted with ether. The ether was washed with water, dried and evaporated, the crude residue was dissolved in dichloromethane and kept at room temperature overnight. The yellow needles of propanediol derivative at position 6(R') were collected and the filtrate was chromatographed and eluted with dichloromethane/acetone (80:1), yellow needles of **4** (1 g, 8%), and **5** (0.8 g, 6%) were obtained. **1,6-Dihydroxy-3-(2,3-dihydroxypropoxy)-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (4)**, m.p. 150–151°. ^{1}H n.m.r. δ 1.69, 1.80, 1.83, 3 x s, 4 × CH_3; 3.38, 4.17, 2 × d, J 7 Hz, C2 and C8-CH_2CH=; 5.18, 5.27. 2 × bt, C2 and C8-CH_2CH=; 3.80, s, OCH_3 6.32, 6.82, 2 × s, H4 and H5; 13.48, s, C1 -OH; 6.48, s, ArOH; substituent group: $OCH_2CHOHCH_2OH$; 4.13, d, J 6Hz, OCH_2; 4.13, m, 2.70, bd, CHOH; 2.10, bt, CH_20H. Mass spectrum *m/z* 484(80%), 441(44), 429(44), 413(100), 367(40), 339(42), 323(23). UV λmax (log ε) 244(4.52), 259(4.47), 316(4.41). IR ν_{max} (cm^{-1}) 3438(b), 1646, 1598, 1461, 1433, 1284, 1197, 1159, 845. (Found: C, 67.1; H, 6.8. $C_{27}H_{32}O_8$ requires C, 66.9; H, 6.7%).

1-Hydroxy-3,6-di(2,3-dihydroxypropoxy)-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (5), m.p. 168–170°. ^{1}H n.m.r. δ 1.68, 1.82, 1.86, 3 × s, 4 × CH_3; 3.39, 4.12, 2 x d, J 7 Hz, C2 and C8-CH_2CH=; 5.40, 5.42, 2 × bt, C2 and C8-CH_2CH=; 3.8 1, s, OCH_3; 6.30, 6.75, 2 × s, H4 and H5; 13.50, s, C 1OH; 6.49, s, ArOH; substituent group: 2 × $OCH_2CHOHCH_2OH$; 4.16, d, J 6 Hz, OCH_2; 4.16, m, 2.65, 2.87, 2 × bd, CHOH; 3.85, 2 × m, 2.00, 2.10, 2 × bt, CH_20H. Mass spectrum *m/z* 558(74%), 515(72), 503(44), 484(94), 441(92), 429(56), 413(100), 339(72). UV λ_{max} (log ε) 254(4.46), 262(4.50), 314(4.36). IR ν_{max} (cm^{-1}) 3425(b), 3364(b), 3264, 1645, 1599, 1279, 1205, 1123, 1044. (Found: C, 64.6; H, 7.0. $C_{30}H_{38}O_{10}$ requires C, 64.5; H, 6.9%).

Preparation of nitrile derivatives (6,7)

A solution of mangostin (2 g, 4.8 mmol) in dimethylformamide (10 ml) and sodium hydride (2 g) in dimethylformamide (5 ml) was stirred at room temperature for 0.5 h then 4-chlorobutyronitrile (1.5 ml, 15 mmol) was added. The reaction mixture was refluxed for 5 h and the reaction mixture was poured into iced water, neutralized with 3 M hydrochloric acid and extracted with dichloromethane. The dichloromethane layer was washed with water and dried over sodium sulfate. The solvent was removed and the residue was chromatographed on silica gel. Elution with the mixed solvent of

hexane / dichloromethane (1:8) gave yellow needles of **6** (1 g, 43%) and **7** (0.25g, 9%). **1-Hydroxy-3,6-di(4-cyanopropoxy)-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (6)** m.p. 133–135°. ^{1}H n.m.r. δ 1.69, 1.79, 1.85, 3 × s, 4 × CH_3; 3.36, 4.13, 2xd, J 7 Hz, C2 and C8-CH_2CH=; 5.18, 5.23, 2 × bt, C2 and C8-CH_2CH=; 3.79, s, OCH_3; 6.30, 6.75, 2 × s, H4 and H5; 13.47, s, C1-OH; substituent group: 2 × $OCH_2CH_2CH_2C{\equiv}N$; 4.18, 2 × t, J 6 Hz, 4.23dd, J 3,11 Hz, OCH_2; 2.22, 2.29, 2 × m, CH_2; 2.64, 2.69, 2 × t, $CH_2C{\equiv}N$. Mass spectrum *m/z* 544(76%), 501(24), 489(30), 477(89), 434(43),422(47), 406(100), 381(16), 323(15). UV λ_{max} (log ε) 247(4.39), 265(4.49), 310(4.32). IR ν_{max} (cm^{-1}) 3400, 1620, 1590, 1450, 1410, 1270, 1190, 1100.

1,3-Dihydroxy-6-(4-cyanopropoxy)-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (7) m.p. 167–170°. ^{1}H n.m.r. δ 1.69, 1.79, 1.83. 3 × s, 4 × CH_3; 3.35, 4.09, 2 × d, J 7 Hz, C2 and C8-CH_2CH=; 5.18, 5.25, 2 × bt, C2 and C8-CH_2CH=; 3.8 1, s, OCH_3; 6.31, 6.83, 2 × s, H4 and H5; 13.45, s, C1-OH; substituent group: $OCH_2CH_2CH_2C{\equiv}N$; 4.17, t, J 6 Hz, OCH_2; 2.21, m, CH_2; 2.62, t, $CH_2C{=}N$. Mass spectrum *m/z* 477(95%), 434(32), 422(52), 406(100), 381(25),323(18). UV λ_{max} (log ε) 247(4.49), 262(4.50), 311(4.39). IR ν_{max} (cm^{-1}) 3380, 1640, 1600, 1460, 1280, 1190, 1110.

Preparation of amino ethyl derivatives (8,9)

A solution of mangostin (5 g, 12 mmol) in dimethylformamide (10 ml) and sodium hydride (5 g) in dimethylformamide (20 ml) was stirred at room temperature for 0.5 h then N,N-diethylaminoethyl chloride hydrochloride (5 g, 29 mmol) was added. The reaction mixture was stirred at room temperature for 20 h then poured into ice water, neutralized with 3 M hydrochloric acid and partitioned with ether. The ether layer was washed with water and dried over sodium sulfate. The solvent was removed and the residue was chromatographed on silica gel. Elution with acetone/methanol (4:1.5) gave a yellow solid **8** (2.4 g, 39%) and **9** (2.25 g, 30%). **1,3-Dihydroxy-6-(N,N-diethylaminoethoxy)-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (8)**, m.p. 142–144°. ^{1}H n.m.r. δ 1.67. 1.74, 1.83, 3 × s, 4 × CH_3; 3.43, 4.05, 2 x d, J 7 Hz, C2 and C8-CH_2CH=; 5.22, 5.28, 2 × bt. C2 and C8-CH_2CH=; 3.76, s, OCH_3; 6.17, 6.70, 2 × s, H4 and H5; 13.76, s, C1–0H; substituent group: $OCH_2CH_2N(CH_2CH_3)2$; 4.15, t, OCH_2; 2.98, t, CH_2; 1. 10, 2.68, 2 × (A_3X_2), J 7 Hz, $N(CH_2CH_3)_2$. Mass spectrum m/z 509(56), 410(46), 367(26), 365(24), 339(74), 323(16), 311(17), 86(100). UV λ_{max} (log ε) 245(4.52), 263(4.51), 315(4.36). IR ν_{max} (cm^{-1}) 3380(b), 3187, 1645, 1596, 1461, 1293, 1202, 842. (Found: C, 70.7; H, 7.8; N, 2.7. $C_{30}H_{39}NO_6$ requires C, 70.7; H, 7.7; N, 2.7%).

1-Hydroxy-3,6-di(N,N-diethylaminoethoxy)-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (9), m.p. 43–45°. ^{1}H n.m.r. δ 1.67, 1.79, 1.85, 3 × s, 4 × CH_3; 3.34, 4.11, 2 × d, J 7 Hz, C2 and C8-CH_2CH=; 5.22, 5.22, 2 × bt, C2 and C8-CH_2CH=; 3.80, *s*, OCH_3; 6.31, 6.73, 2 x s, H4 and H5; 13.41, s, -OH; substituent group: 2 × $OCH_2CH_2N(CH_2CH_3)_2$; 4.16, 2 × t, 2 × OCH_2; 2.93, 2.97, 2 × t, 2 × CH_2; 1.08, 2.66, A_3X_2, J 7 Hz, $N(CH_2CH_3)_2$. Mass spectrum m/z 608(7), 100(39), 86(100). UV λ_{max}(log ε) 245(4.48), 262(4.51), 313(4.38). IR ν_{max} (cm^{-1}) 1644, 1597, 1462, 1430, 1275, 1202. (Found: M^+, 608.3742. $C_{36}H_{52}N_2O_6$ requires M^+, 608.3824).

Preparation of amino derivative (10)

A solution of mangostin (2.5 g, 6 mmol) in dimethylformamide (5 ml) and sodium hydride (2.5 g) in dimethylformamide (10 ml) was stirred at room temperature for 0.5 h then N,N-dimethylaminoethyl chloride hydrochloride (2.25 g, 15.73 mmol) was added. The reaction mixture was stirred at room temperature for 10 h then poured into ice water, neutralized with 3 M hydrochloric acid and partitioned with dichloromethane. The organic layer was washed with water and dried over sodium sulfate. The solvent was removed and the residue was chroma-

tographed on silica gel. Elution with dichloromethane / methanol (95:5) gave compound **10** as a yellow solid (0.472 g, 14%). **1,3-Dihydroxy-6-(N,N-dimethylaminoethoxy)-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (10)**, m.p. 180–182°. ^{1}H n.m.r. δ 1.66, 1.68, 1.78, 1.83,4 × s, 4 × CH_3; 3.29, d, 4.06, 2 × d, J 7 Hz, C2 and C8-CH_2CH=; 5.17, 5.24, 2 × bt, C2 and C8-CH_2CH=; 3.79, s, OCH_3; 6.22, 6.24, 2 × s, H4 and H5; 5.25, 2 × bt, C2 and C8-CH_2CH=; 3.81, s, OCH_3; 6.23, 6.82, 2 × s, H4 and H5; 13.45, s, C1-OH; substituent group: $OCH_2CH_2N(CH_3)_2$; 4.24, t, OCH_2; 3.00, t, CH_2; 2.55, s, $N(CH_3)_2$. Mass spectrum *m/z* 481(35), 409(14), 72(83), 58(100).

Preparation of amino derivative 11

Derivative **11** was prepared as for compound 10 except N,N-dimethylaminopropyl chloride was used. Separation on silica gel with dichloromethane/methanol (75:25) gave **11** in 26% yield. **1,3-Dihydroxy-6-(N,N-dimethylaminopropoxy)-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (11)**; m.p. 89–92. ^{1}H n.m.r. δ 1.68, 1.79, 1.89, 3 × s, 4 × CH_3; 3.30, d, 4.08, 2 x d, J 7 Hz, C2 and C8-CH_2CH=; 5.10, 5.25, 2 × bt, C2 and C8-CH_2CH=; 3.81, s, OCH_3; 6.23, 6.82, 2 × s, H4 and H5; 13.45, s, Cl-OH; substituent group: $OCH_2CH_2CH_2N(CH_3)_2$; 4.13, t, OCH_2; 2.30, t, CH_2; 3.00, s, CH_2; 2.67, s, $N(CH_3)_2$. Mass spectrum m/z 495(29), 409(19), 86(51), 58(100). UV λ_{max} (logε) 350(3.82), 314(4.38), 262(4.47), 246(4.51). IR ν_{max} (cm^{-1}) 3400, 2920, 1640, 1600, 1460, 1280, 1200, 1110.

Preparation of derivatives (12, 13)

Compounds 12 and 13 were prepared from the intermediate epoxide derivatives which were prepared by reaction of mangostin (5 g, 0.012 mol) in demithylformamide (20 ml), stirred with sodium hydride (2 g) in dimethylformamide at room temperature for 0.5 h, then 1-chloro-2,3-epoxypropane (2.5 ml, 0.031 mol) was added. After stirring for 6 h the mixture was added to ice water, neutralized with 3M hydrochloric acid and the crude product extracted into ether. Chromatography on silica gel and elution with light petroleum/dichloromethane (4:5) gave the epoxide derivatives.

Diethylamine, dimethylamine and isopropylamine (0.5 ml) were individually added to the suspensions of epoxide derivatives (500 mg) in methanol (5 ml) and the reaction mixture was refluxed for 4–6 h. The solutions were evaporated to remove the solvent and excess amine. The residue was chromatographed on silica gel. Elution with the mixed solvent of dichloromethane and methanol gave the pure products **12** (300 mg, 58%), and **13** (356 mg, 63%), respectively. **1,3-Dihydroxy-6-(2-hydroxy-3-N,N-dimethylaminopropoxy)-7-methoxy-2,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (12)**, m.p. 148–149°. ^{1}H n.m.r. ($CDCl_3$ + $DMSOd_6$) δ 1.69, 1.81, 1.82, 3 × s, 4 × CH_3; 3.35, 4.08, 2 × d, J 7Hz, C2 and C8-CH_2CH=; 5.20, 5.26, 2 × bt, C2 and C8-CH_2CH=; 3.80, s, OCH_3; 6.29, 6.80, 2 × s, H4 and H5; 13.42, s, C1-OH; substituent group: $OCH_2CHOHCH_2N(CH_3)_2$; 4.04, m, OCH_2; 4.14, m, CHOH; 2.52, 2.62, 2 × dd, CH_2; 2.39, s, $N(CH_3)_2$. UV λ_{max} (log ε) 244(4.53), 259(4.49), 315(4.41). IR ν_{max} (cm^{-1}) 3306(b), 1644, 1596, 1571, 1465, 1283, 845. (Found: M^+, 539.2812. $C_{31}H_{41}NO_7$ requires M^+; 539.2882). **1,3-Dihydroxy-6-(2-hydroxy-3-N-isopropylaminopropoxy)-7-methoxy-2,8-bis (3-methylbut-2-enyl-9*H*-xanthen-9-one (13)**, m.p. 102–104°. ^{1}H n.m.r. δ 1.68, 1.79, 1.83, 3 × s, 4 × CH_3; 3.35, 4.09, 2 × d, J 7 Hz, C2 and C8CH_2CH=; 5.20, 5.23, 2 × bt, C2 and C8-CH_2CH=; 3.80, s, OCH_3 6.33, 6.80, 2 × s, H4 and H5; 13.61, s, C1-OH; substituent group: $OCH_2CHOHCH_2NHCH(CH_3)_2$; 4.05, m, OCH_2; 4.19, m, CHOH and CH; 2.80–3.00, m, CH_2; 1.18, d, $(CH_3)_2$. UV λ_{max} (log ε) 356(3.76), 315(4.36), 263(4.45), 246(4.47). IR ν_{max} (cm^{-1}) 3290(b), 2930, 1650, 1600, 1570, 1455, 1290, 1200, 1115.

Preparation of derivative 14

Dimethylamine and isopropylamine (0.5 ml) were individually added to the suspensions of epoxide derivatives (500 mg) in methanol (5 ml) and the reaction mixture was refluxed for 4–6 h. The solutions were evaporated to remove the solvent and excess amine. The residue was chromatographed on silica gel. Elution with dichloromethane and methanol gave pure **14** (417 mg, 68%). **1-Hydroxy-3,6-di(2-hydroxy- 3-N-isopropylaminopropoxy)-7-methoxy-2,8- bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one (14)**, m.p. 73–76°. ^{1}H n.m.r. δ 1.66, 1.68, 1.78, 1.84, 4 × s, 4 × CH_3; 3.33, 4.09, 2 × d, J 7 Hz, C2 and C8-CH_2CH=; 5.18, 5.22, 2 × bt, C2 and C8-CH_2CH=; 3.79, s, OCH_3; 6.23, 6.63, 2 × s, H4 and H5; substituent group: 2 × $OCH_2CHOHCH_2NHCH(CH_3)_2$; 4.03, m, OCH_2; 4.15, m, CHOH; 2.80–3.00, m, 2 × CH_2 and CH; 1.13, 1.14, 2 × d, $(CH_3)_2$. UV λ_{max} (log ε) 356(3.80), 310(4.33), 264(4.52), 248(4.53), 226(4.04). IR ν_{max} (cm^{-1}) 3400, 2915, 1650, 1600, 1470, 1380, 1205, 1115.

Preparation of derivative 15

A solution of isomangostin[19] (1 g, 2.4 mmol) in dimethylformamide (3 ml) was stirred with sodium hydride (1 g) in dimethylformamide (5 ml) at room temperature for 0.5 h then N,N-dimethylaminoethychloride hydrochloride (713 mg, 4.9 mmol) was added. The reaction mixture was refluxed for 5 h then poured into ice water and partitioned with dichloromethane. The aqueous layer was neutralized with 3M hydrochloric acid and partitioned with dichloromethane. The dichloromethane layer was washed with water and dried over sodium sulfate. The solvent was removed and the residue was chromatographed on silica gel. Elution with dichloromethane and methanol (9:1) gave yellow solid of **15** (659 mg, 56%). **5-Hydroxy-8-methoxy-9-(2-N,N-dimethylaminoethoxy)-7-(3-methylbut-2-enyl)-2,2-dimethylpyrano[3,2-*b*]xanthen-6-one (15)**, m.p. 154–157°. ^{1}H n.m.r. δ 1.67, 1.84, 2 × s, 2 × CH_3; 1.38, s, $OC(CH_3)_2$; 4.12, d, C11-CH_2CH=; 5.23, bt,C11-CH_2CH=; 1.84, 2.71, 2 × t, J 7 Hz, ArCH_2CH_2; 3.79, s, OCH_3; 6.22, 6.72, 2 × s, H10, H12; 13.79, s, C5-OH; substituent group: $OCH_2CH_2N(CH_3)_2$; 4.20, t, J 7 Hz, OCH_2, 2.88, t, J 7 Hz, CH_2; 2.40, s, $N(CH_3)_2$. UV λ_{max} (log ε) 358(3.92), 318(4.39), 262(4.50), 246(4.47). IR ν_{max} (cm^{-1}) 3420, 2920, 1640, 1600, 1470, 1280, 1200, 815.

Preparation of derivative 16

A solution of isomangostin (1 g, 2.4 mmol) in dimethylformamide (3 ml) was stirred with sodium hydride (1 g) in dimethylformamide (5 ml) at room temperature for 0.5 h then N,N-dimethylaminopropyl chloride hydrochloride (800 mg, 5 mmol) was added. The reaction mixture was refluxed for 6 h then poured into ice water and partitioned with dichloromethane. The aqueous layer was neutralized with 3 M hydrochloric acid and partitioned with dichloromethane, which was washed with water and dried over sodium sulfate. The solvent was removed and the residue purified by silica gel chromatography; elution with dichloromethane and methanol (85:15) gave a yellow solid of **16** (210 mg, 21%). **5-Hydroxy-8-methoxy-9-(3-N, N-dimethylaminopropoxy)-7-(3methyl-but-2-enyl)-2,2-dimethylpyrano[3,2-*b*]xanthen-6-one (16)**, m.p. 154157°. ^{1}H n.m.r. δ 1.68, 1.83, 2 × s, 2 × CH_3; 1.38, s, $OC(CH_3)_2$; 4.12, d, C11-CH_2CH=; 5.25, bt, C11 – CH_2CH=; 1. 83, 2.71, 2 × t, J 7 Hz, ArCH_2CH_2; 3.80, s, OCH_3; 6.22, 6.77, 2 × s, H10, H12; 13.78, s, C5-OH; substituent group: $OCH_2CH_2CH_2N(CH_3)_2$; 4.15, t, J 7 Hz, OCH_2, 2.12, m, CH_2; 2.62, t, J 7 Hz, CH_2N; 2.35, s, $N(CH_3)_2$. UV λ_{max} (log ε) 354(3.94), 318(4.37), 262(4.49), 245(4.48). IR ν_{max} (cm^{-1}) 3420, 2940, 1640, 1600, 1470, 1400, 1280, 1200, 1160, 1120, 820

Preparation of derivative 17

3-Isomangostin (1 g, 2.4 mmol) in methanol (8 ml) was added to a solution of sodium (0.11 g, 4.8 mmol) in methanol (4 ml) then

1-chloro-2,3-epoxypropane (0.4 ml, 4.9 mmol) was added. After being refluxed for 15 h the solution was evaporated and the residue partitioned between dichloromethane and dilute acid. Chromatography on silica gel, elution with dichloromethane and dichloromethane/acetone gave the epoxide (0.09 g, 8%). The major product was the derived methoxy alcohol (0.41 g, 33%).

Dimethylamine (1 ml) was added to the suspension solution of epoxide derivative (500 mg, 1.1 mmol) in methanol (5 ml) was refluxed for 7 h. The reaction mixture was evaporated to remove the solvent and excess amine. The residue purified on silica gel by elution with dichloromethane and methanol (9:1) to give a yellow solid of **17** (475 mg, 87%). **5-Hydroxy-8-methoxy-9-(2-hydroxy-3-N,N-dimethylaminopropoxy)7-(3-methylbut-2-enyl)-2,2-dimethylpyrano[3,2-*b*]xanthen-6-one (17)**, m.p. 192–194°. ^{1}H n.m.r. δ 1.68, 1.84, 2 × s, 2 × CH_3; 1.37, s, $OC(CH_3)_2$; 4.12, d, J 7 Hz, C11-CH_2CH=; 5.23, bt, C11-CH_2CH=; 1.84, 2.70, 2 × t, J 7 Hz; $ArCH_2CH_2$; 3.79, s, OCH_3; 6.22, 6.74, 2 × s, H10, H12; 13.68, s, C5-OH; substituent group: $OCH_2CHOHCH_2$ $N(CH_3)_2$; 4.20, 4.08, 2 × m, OCH_2; 4.45, m, CHOH; 2.98, m, CH_2; 2.70, s, $N(CH_3)_2$. Mass spectrum m/z 511(12), 102(20), 58(100). UV λ_{max} (logε) 318(4.40), 262(4.52), 246(4.49). IR ν_{max} (cm^{-1}) 3450, 2980, 2920, 1640, 1600, 1220, 1080, 1040.

Preparation of derivative 18

Isopropylamine (1 ml) was added to a suspension of epoxide derivative (500 mg, 1.1 mmol) in methanol (5 ml) and refluxed for 8 h. The reaction mixture was evaporated to remove the solvent and excess amine. The residue was separated on silica gel. Elution with dichloromethane and methanol (9:1) gave 18 as a yellow solid (440 mg, 76%). **5-Hydroxy-8-methoxy-9-(2-hydroxy-3-N-isoproylaminopropoxy)-7(3-methylbut-2-enyl)-2,2-dimethylpyrano[3,2-*b*]xanthen-6-one (18)**, m.p. 148–149°. ^{1}H n.m.r. δ 1.67, 1.84, 2 × s, 2 × CH_3; 1.36, s, $OC(CH_3)_2$; 4.13, d, J 7 Hz, C11-CH_2CH=; 5.25, bt, C11-CH_2CH=; 1.85, 2.72, 2 × t, J 7 Hz, $ArCH_2CH_2$; 3.82, s, OCH_3; 6.24, 6.78, 2 × s, H10, H12; 13.75, s, C5-OH; substituent group $OCH_2CHOHCH_2$ $N(CH_2CH_3)_2$; 4.13, m, OCH_2; 4.13, m, CHOH; 2.70, m, CH_2; 1.15, 2.70, 2 × (A_3X_2), $N(CH_2CH_3)_2$ Mass spectrum m/z 539(31%), 410(36), 381(29), 367(35), 325(29), 297(40), 86 (100). IR ν_{max} (cm^{-1}) 3450, 2960, 2820, 1640, 1600, 1220, 1200, 1090, 1040.

Preparation of compound 19

3-Isomangostin (2g, 4.8 mmol) was treated with 4-chlorobutyronitrile in the same manner as preparation of compounds 6, **7** to give compound 19 in 50% yield (yellow needles, 1.06 g). **5-hydroxy-8-methyl-(3-cyanobutoxy)-7-(3-methylbut-2-enyl)-2,2-dimethylpyranol[3,2-*b*]xanthen-6-one (19)**, mp. 153–158° C. ^{1}H nmr δ 1.68, 1.87, 2× s, 2x CH_3; 1.37, s, $OC(CH_3)_2$; 4.04, d, *J*7Hz, C11-CH_2CH=; 5.25, bt, C11-CH_2CH=; 1.87, 2.72, 2 × t, *J*7Hz, Ar-CH_2CH_2; 3.78, s, OCH_3; 6.23, 6.75, 2 × s, H10, H12; 13.75, s, C5-OH. Substituent group: $OCH_2CH_2CH_2C$=N 4.21, t, OCH_3, 2.26, m, CH_2; 2.66, t, CH_2=N. Mass spectrum m/z 477(47%), 462(10), 434(100), 422(7). UV λ_{max} (logε) 247(4.40), 263(4.50), 316(4.36). IR ν_{max} 3400, 2220, 1620.

Preparation of compound 20

Bicyclomangostin (20) was prepared as previously reported[20].

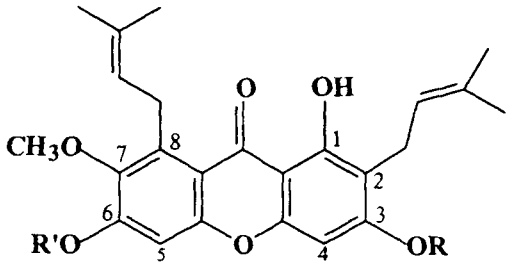
RESULTS

Two main strategies were followed to prepare structural derivatives of mangostin. Firstly, modification of the two reactive phenolic hydroxyl groups at positions 3 and 6 was undertaken to produce compounds **2 – 14**. Functionalisation of the phenolic hydroxyl groups was carried out to block either one or both groups and also possibly alter the solubility characteris-

tics of the molecule. Derivatives prepared include methyl and acetate derivatives (**2–3**), alcohols (**4,5**), nitriles (**6,7**) and amines (**8–14**). The second strategy involved modification of the prenyl side chain of mangostin since this may be involved in its biological activity. The prenyl side chain at C-2 can react easily with the *ortho*-hydroxy group at C-3 to produce the cyclisation product illustrated by isomangostin[19]. Various amine derivatives were then prepared at the C-6 hydroxyl to produce compounds **15–19**. Compound **20** involved cyclisation of both prenyl side chains and bonding of the oxygen function at C-9[20]. The structure of all derivatives was confirmed by a combination of n.m.r. spectroscopy, mass spectrometry and UV and IR spectroscopy.

Initial screening of compounds for antioxidant activity used the *in vitro* oxidation of LDL mediated by Cu^{++}[14]. Initial concentration for the screening experiments was chosen at 10 μM since at this concentration mangostin was previously shown to have some antioxidant activity[8]. The results of screening are shown in table I and II as % inhibition of lipid hydroperoxide formation at 90 minutes. Structural modification had a major effect on antioxidant activity in some cases. Methylation of the C-3 and C-6 phenolic hydroxyls substantially decreased antioxidant activity. Preparation of either the acetate, alcohol or nitrile derivatives at C-3 and/or C-9 resulted in nearly complete loss of antioxidant activity. In contrast, the diethylaminoethoxy derivatives **8** and **9** showed a marked increase in antioxidant activity. Cyclisation of either one or both of the prenyl side chains appeared to have little impact on the antioxidant activity of the mangostin molecule. In order to make a more detailed study of the antioxidant activity of these compounds a small selection was chosen for detailed analysis. Compounds **8** and **9** were chosen because they showed the greatest activity in the screening studies. Compound **20** was also studied because it was the greatest structural alteration to mangostin while still maintaining antioxidant activity.

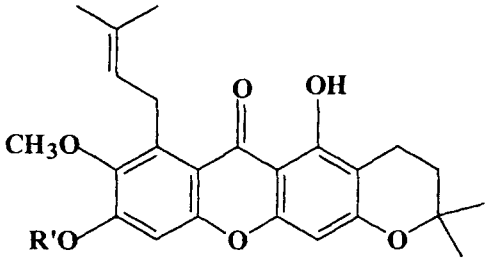
TABLE I Structures and antioxidant activity of mangostin and derivatives. All compounds were screened for antioxidant activity at 10 μM, oxidation of LDL (0.1 mg/ml protein) was initiated with 5 μM Cu^{++} at 37°C. Lipid hydroperoxide formation was measured at 90 min and values expressed as % inhibition compared to control oxidations. Values are the mean ± SEM based on between 3 to 6 experiments



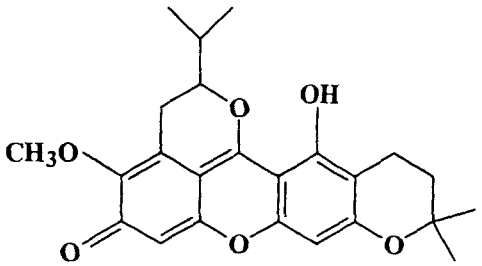
Compound	*R'*	*R*	*% Inhibition*
1	H	H	23 ± 6
2	CH_3	CH_3	11 ± 5
3	$COCH_3$	H	–
4	H	$CH_2CHOHCH_2OH$	–
5	$CH_2CHOHCH_2OH$	$CH_2CHOHCH_2OH$	–

6	$CH_2\ CH_2\ CH_2CN$	$CH_2\ CH_2\ CH_2CN$	5 ± 2
7	$CH_2\ CH_2\ CH_2CN$	H	3 ± 1
8	$CH_2\ CH_2\ N(CH_2\ CH_3)_2$	H	43 ± 8
9	$CH_2CH_2N(CH_2CH_3)_2$	$CH_2CH_2N(CH_2CH_3)_2$	44 ± 5
10	$CH_2CH_2N(CH_3)_2$	H	24 ± 6
11	$CH_2CH_2CH_2\ N(CH_3)_2$	H	16 ± 7
12	$CH_2CHOHCH_2\ N(CH_2CH_3)_2$	H	10 ± 4
13	$CH_2CHOHCH_2\ NHCH\ (CH_3)_2$	H	16 ± 8
14	$CH_2CHOHCH_2\ NHCH\ (CH_3)_2$	$CH_2CHOHCH_2\ NHCH\ (CH_3)_2$	29 ± 4

TABLE II Structures and antioxidant activity of the cyclised derivatives of mangostin. Details are the same as those given in table I



Compound	*R'*	*% Inhibition*
15	$CH_2CH_2N(CH_3)_2$	27 ± 5
16	$CH_2CH_2CH_2N(CH_3)_2$	24 ± 7
17	$CH_2CHOHCH_2N(CH_3)_2$	11 ± 3
18	$CH_2CHOHCH_2N(CH_2CH_3)_2$	38 ± 6
19	$CH_2CH_2CH_2CN$	9 ± 3



Compound **20.** % Inhibition = 28 ± 5

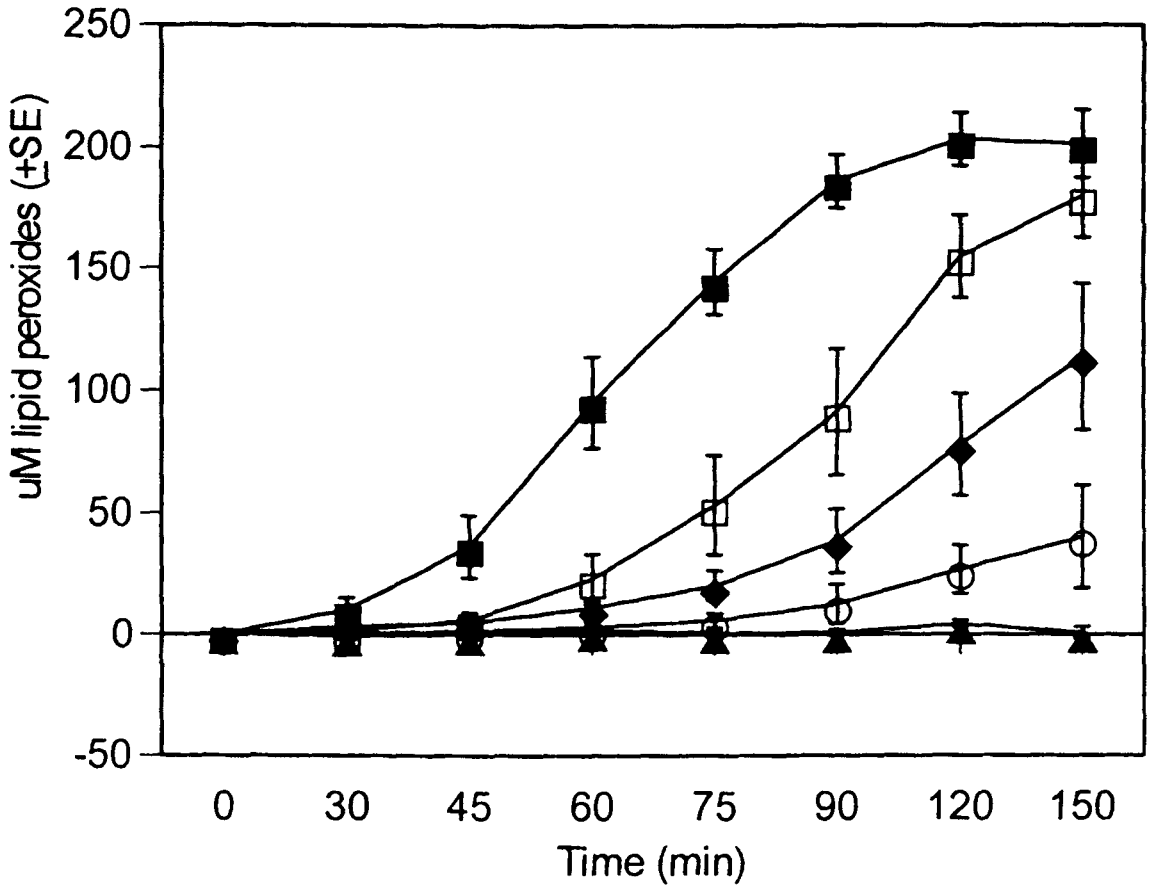


FIGURE 1 Time course of LDL oxidation initiated with 5 μM Cu^{++}showing the formation of lipid hydroperoxides (LOOH) in the presence of various test compounds (50 μM); mangostin **1** (□), compound **8** (•), compound **9** (▲), compound **20** (♦) or vehicle control (ethanol 10μl, ■). Data is presented as the mean ± standard error of 5 separate experiments

Figure 1 shows the kinetics of lipid hydroperoxide formation in Cu^{++}-mediated LDL oxidation in the presence of 50 μM of the test compounds. At this concentration compound **9** totally prevented lipid hydroperoxide formation over 150 min. Each derivative tested was more potent at preventing oxidation than mangostin itself. In order to gain a better understanding of the dose response effects of these compounds in this assay, compounds were tested at 1, 10 and 50 μM. Figure 2 shows the dose response curves obtained for each compound where antioxidant activity is expressed as % inhibition of lipid hydroperoxide formation compared to control at 90 min. The IC_{50} concentration for all three derivatives of mangostin is about 10 μM and for mangostin itself the IC_{50} concentration is 50 μM. It is noted that the antioxidant activity of the derivatives 8,9 and 20 at 10 μM was slightly higher in the dose response study compared to the screening assay and this may be due to the fact that they were carried out at different times using different LDL preparations.

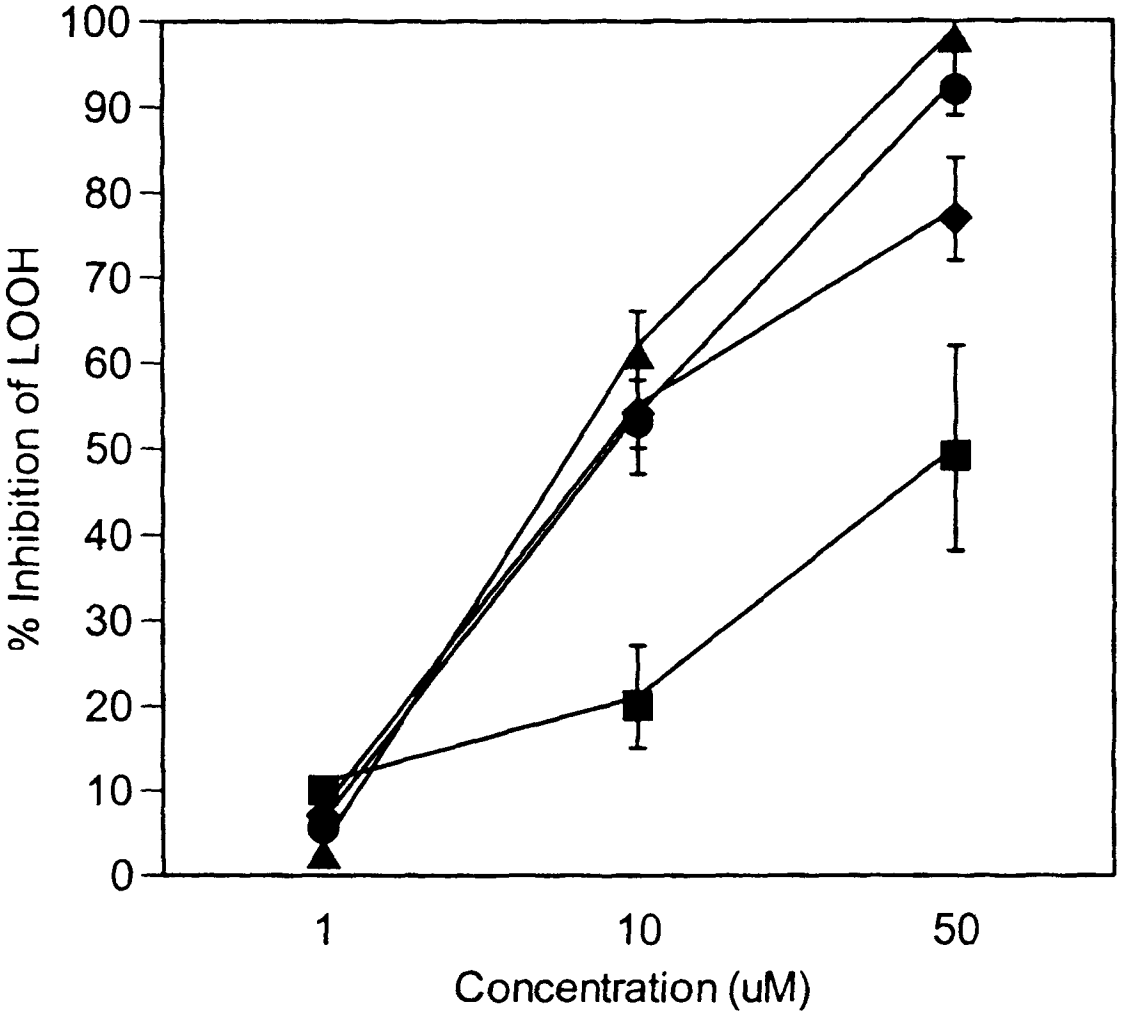


FIGURE 2 Dose response curves for the inhibition of lipid hydroperoxide (LOOH) formation in Cu^{++}-mediated LDL oxidation by mangostin (■), compound **8** (•), **9** (▲) and **20** (♦). Results are expressed as % inhibition of control oxidation and are the mean and standard error of three experiments

As a means to determine possible antioxidant mechanism of these compounds, the endogenous levels of α-tocopherol in LDL were measured during Cu^{++}-mediated oxidation in the presence or absence of 50 μM of compound **9** (the most active antioxidant derivative). Figure 3 shows that the consumption of α-tocopherol was similar between the control and treatment LDL samples. Since metal ion-mediated oxidation of LDL is a complex process dependent on a number of factors such as metal ion reduction and peroxyl radical formation by Fenton-type reactions[21], we decided to investigate another model of lipoprotein oxidation not dependent on metal ions. The thermally unstable azo compound, AAPH, decomposes at a steady rate to

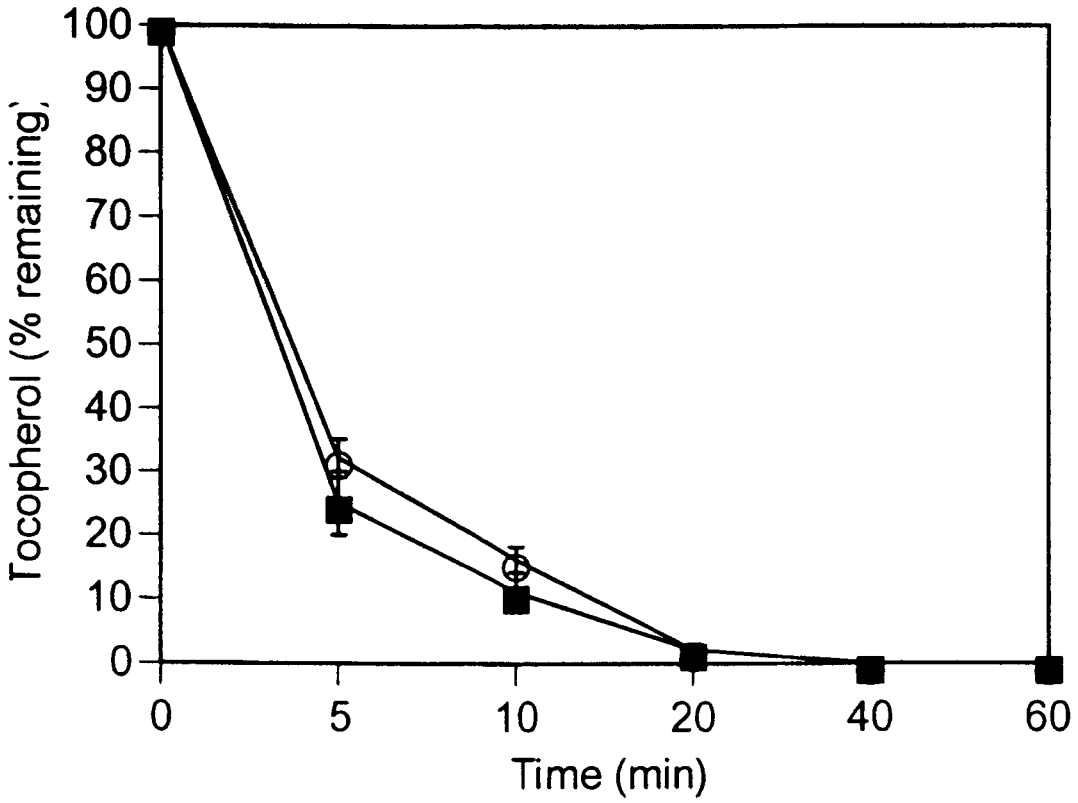


FIGURE 3 The effect of 50 μM of compound 9 on the consumption of α-tocopherol in LDL after initiation of oxidation with 5 μM Cu++. Open circles represent LDL with 50 μM compound 9 and closed squares represent LDL with vehicle (ethanol control). Initial concentration of α-tocopherol in LDL was 3 μM. Results are the mean and SEM of three experiments

produce peroxyl radicals that can oxidise LDL[22]. Figure 4 shows lipid hydroperoxide formation in response to 4mM AAPH in the absence and presence of 1 μM concentrations of mangostin and the derivatives **8, 9** and **20**. These compounds are much more effective at preventing AAPH induced oxidation of LDL than metal ion oxidation. At 1 μM concentrations these compounds have almost no inhibitory effect against Cu^{++} induced LDL oxidation (Figure 2). The relative potency of the compounds appears similar in both oxidation assays with compound **9** being most active and mangostin being the least active.

To determine if these compounds could inhibit lipoprotein oxidation in a more physiological setting, compounds were added to diluted serum, which was oxidised with Cu^{++}. Figure 5 shows the time course of serum lipoprotein oxidation and indicates that compound **9** can effectively inhibit this process at a concentration as low as 1 μM.

Determination of the relative solubilities of mangostin and compound **9** was undertaken by determining their partition coefficients in octanol / water mixtures. Derivative **9** had a lower K_{part} (3.5) than mangostin (13), indicating that it was more water soluble than mangostin.

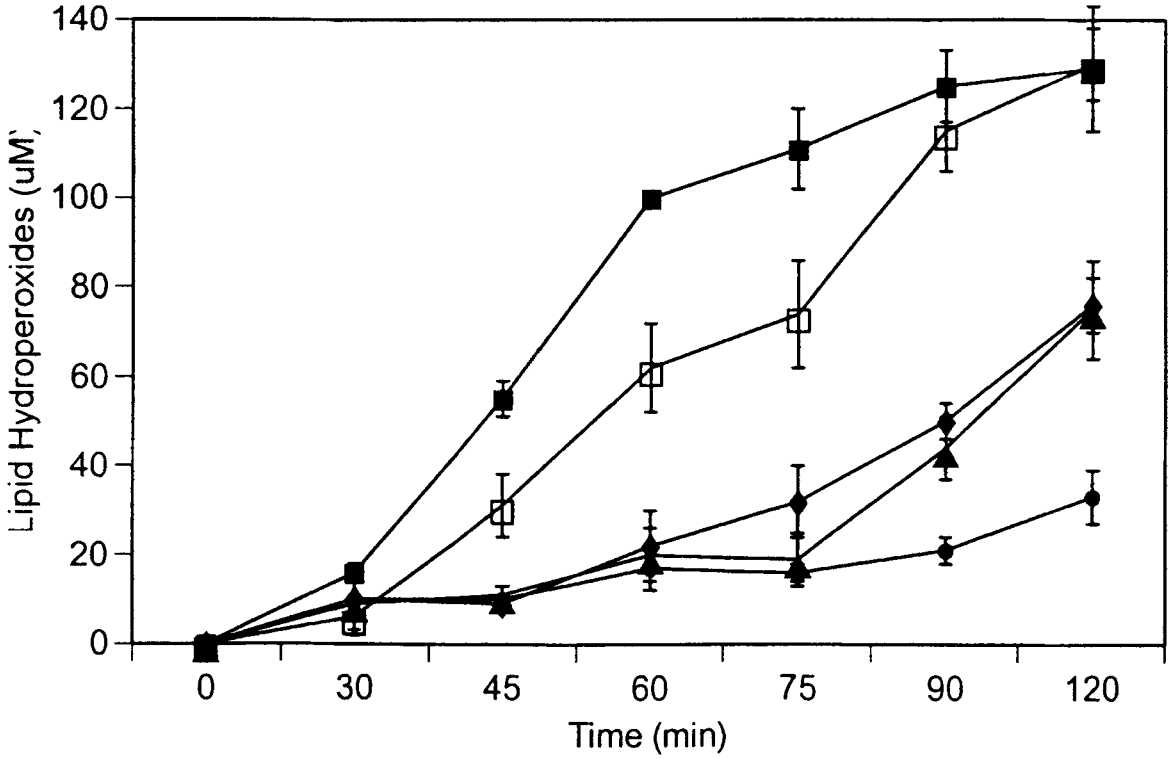


FIGURE 4 The effect of various test compounds (1 μM) on the formation of lipid hydroperoxides (LOOH) during LDL oxidation by AAPH (4 mM, 37°C). Control oxidation (■), mangostin (□), compound **8** (•), **9** (▲) and **20** (♦), results are the mean and standard error of three experiments

DISCUSSION

The results of this study show that structural modification to the mangostin molecule can have a profound effect on antioxidant activity. Our principal findings were that derivatisation of the C-3 and C-6 hydroxyl groups with either methyl, acetate, propane diol or nitrile substantially reduced antioxidant activity. In contrast, derivatisation of C-3 and C-6 with aminoethyl derivatives enhanced antioxidant activity indicating that the free hydroxyl groups at the 3 and 6 position are not necessary for antioxidant activity. Cyclisation of the prenyl chains had little influence on antioxidant activity.

There are no "structure -activity" studies published on the xanthones in relation to antioxidant activity. However in another class of plant derived polyphenolic compounds, namely the flavonoids, much work has been carried out[4–7]. While the specific mode of inhibition of lipid peroxidation by these polyphenolics is not entirely clear, they may act by chelation of copper ions and by acting as hydrogen-donating radical scavengers[23]. There are thought to be three main structural features that are important for antioxidant activity of the flavonoids. These are; the presence of *o*-dihydroxy structure in the B ring which confers stability to the radical form and participates in electron delocalisation; a 2,3 double bond in conjugation with the 4-oxo func-

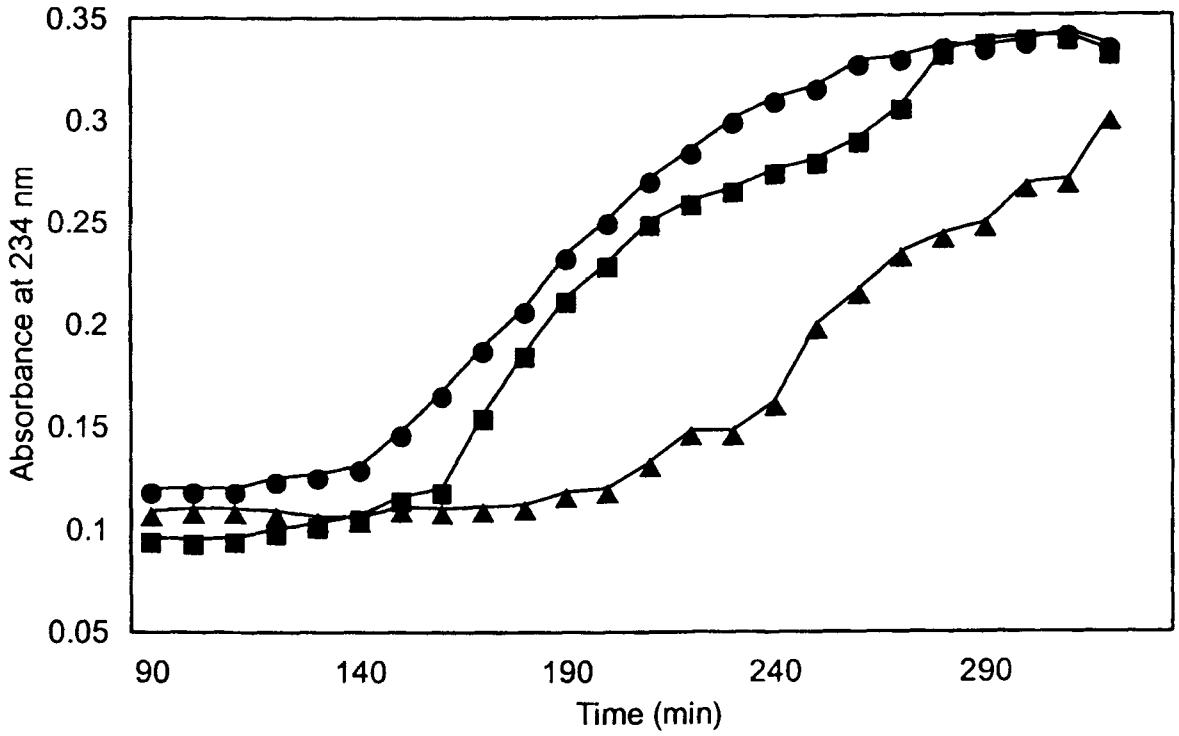


FIGURE 5 The effect of 1 μM compound **9** (▲), and mangostin (■) on lipid peroxidation (indicated by conjugated diene absorption at 234 nm) in diluted serum. Control oxidation is represented by (•), results are representative of three experiments

tion of the C ring contributing to electron delocalisation from the B ring; and the presence of 3- and 5- OH groups in the A ring[23,24]. It is difficult to compare antioxidant activity of the flavonoids with the mangostin xanthones because of differences in methods often used to assess antioxidant activity. However a comparison with several published studies using Cu^{++} mediated LDL oxidation suggest that the prenylated xanthone derivatives studied have similar activity to some flavonols such as quercetin[25] and to some prenylated flavones isolated from *Artocarpus heterophyllus*[26]. One strikingly different feature of our results compared to previously published studies of the flavonoids is that the mangostin compounds are more effective at inhibiting AAPH -induced peroxidation than Cu^{++}mediated peroxidation. Previous studies with flavonoids suggest they inhibit metal ion -induced peroxidation more effectively than AAPH-induced oxidation[24,27].

Given that the mangostin compounds lack any o-dihydroxy functions it is surprising that they display similar antioxidant activity to the flavonoids. The oxo function bounded by a OH at position 1 is a similar structural feature to that found in flavonols such as quercetin, formation of a phenoxyl radical at the 1 position would presumably be stabilised by the neighbouring oxo function. In compound **20** where the oxygen neighbouring C-1 has been cyclised with the prenyl chain, delocalisation of any radical formed on the C-1 hydroxyl could still be possible through the oxo function at C-6. Hence compound **20** still maintains considerable antioxidant activity.

The striking differences in antioxidant activity between mangostin and the derivatives **8,9** and **20** are well illustrated in the time-course experiments in figures 2 and 4, which show the compound **9** having the most effective antioxidant activity against both Cu^{++} and AAPH-initiated peroxidation. The precise reason for this increased activity is not clear but determination of partition coefficients indicate that this compound (**9**) is more water soluble than mangostin and therefore may be better able to reach the site of lipid peroxidation, perhaps at the surface of the lipoprotein particles and remove or trap peroxyl radicals. This may particularly be the case with the water soluble AAPH initiated oxidation. The sparing of α-tocopherol does not seem to be a major factor in the inhibition of LDL peroxidation since the rate of consumption was not different between the presence or absence of compound **9** (figure 3).

For a compound to be considered as an antioxidant it should fulfill two basic requirements. Firstly, when present at low concentrations relative to the substrate being oxidised (in this case polyunsaturated fatty acids in the form of cholesteryl esters in LDL) it can delay or prevent free radical-mediated oxidation of the substrate, and secondly, the resulting radical formed after scavenging must be stable[28]. By these criteria mangostin and the derivatives **8,9** and **20** would certainly be considered as useful antioxidants. The model in which we have studied their activity is also very appropriate given that LDL is a major target for oxidative modification in the body and is thought to play a key role in atherogenesis[1–3]. Moreover, compound **9** appears to be an effective antioxidant at low concentration (1μM) in human plasma, whereas mangostin itself offers little protection at this concentration. What is not known is the extent to which these compounds may be absorbed and metabolised in the body. There is some information regarding the absorption of the flavonoids, which suggests they may be found in the circulation at concentrations capable of exerting a physiological effect[29]. Further work is required to examine the absorption and metabolism of mangostin and its various derivatives, but the results outlined here demonstrate that some structural modifications to mangostin are capable of enhancing its antioxidant activity.

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