The Structure of Garcinone E

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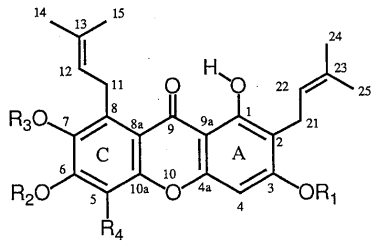
As part of a survey of anti-tumorpromotive chemicals, garcinone E (1) and nine known xanthones, 8-deoxygartanin, gartanin, xanthone I, β-mangostin, garcinone B, 6-deoxy-γ-mangostin, 1,5,8-trihydroxy-3-methoxy-2-(3-methyl-2-butenyl)xanthone, γ-mangostin and α-mangostin, together with egonol, were isolated from the fruit hulls of *Garcinia mangostana* L. collected in Thailand. The structure of garcinone E was elucidated as 1,3,6,7-tetrahydroxy-2,5,8-tri-(3-methyl-2-butenyl)xanthone by spectroscopic methods, including the application of a number of two dimensional (2D) NMR techniques (^{1}H–^{1}H, ^{13}C–^{1}H correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY) and correlation *via* long-range coupling (COLOC).

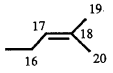
Keywords garcinone E; structure elucidation; xanthone; 1,3,6,7-tetrahydroxy-2,5,8-tri-(3-methyl-2-butenyl)xanthone; *Garcinia mangostana*; egonol

Nine minor polyoxygenated xanthones have been isolated by other workers along with the major products, α-mangostin and γ-mangostin, from the fruit hulls of *Garcinia mangostana*.[1,2] Two other minor polyoxygenated xanthones were also isolated from the leaves.[1b] We found that α- and γ-mangostins (**2** and **3**) weakly inhibited irritation of mouse ear induced by teleocidin, a tumor promoter.[3] From this viewpoint, we have reinvestigated the constituents of the fruit hulls. Recently, Banerji *et al.* isolated a new minor xanthone, garcinone E (**1**), having the molecular formula $C_{28}H_{32}O_4$ (*m/z*, 464) as an amorphous powder. A tentative structure was proposed based on spectral data for the acid-catalyzed cyclization product of this compound (**1**).[4] The present paper reports the isolation and structure confirmation of crystalline **1** from the fruit hulls, along with other known xanthones and egonol.[5]

Results and Discussion

The fruit hulls of *G. mangostana* were extracted with benzene according to the original method.[2a] A crude yellow crystalline mass (A, 17 g), the residue of the benzene mother solution (B, 4.5 g) and the methanol extract (C, 28.7 g) obtained by the Soxhlet method were obtained from dried powder of the fruit hulls (300 g). Fraction A and B exhibited weak inhibitory activity as described above, but no activity was found in fraction C. Fraction A provided pure crystalline α-mangostin (**2**) and γ-mangostin (**3**). Fraction B, after SiO_2 column chromatographic separation, afforded garcinone E (**1**), along with ten known compounds as shown in Table I.



R_1=R_2=R_3=H, R_4=  garcinone E (**1**)

R_1=R_2=CH_3, R_3=H, R_4=  (**1a**)

R_1=R_2=R_3=CH_3, R_4=  (**1b**)

R_1=R_2=R_4=H, R_3=CH_3 α-mangostin (**2**)

R_1=R_2=R_3=R_4=H γ-mangostin (**3**)

R_1=R_3=CH_3, R_2=R_4=H β-mangostin (**4**)

The ^{1}H-NMR spectrum of **1** showed the presence of six methyl groups on vinyl carbon between δ 1.71 and 1.88 (s, CH_3 × 6). The signals due to three benzylic methylenes at

TABLE I. Constituents from Benzene Extract of Mangosteen Hulls[a]

Fraction[b]	Compound	Yield (mg)	Ref.
1	8-Deoxygartanin	32[c]	6
2	Gartanin	71[c]	6
3	Xanthone I	26[c]	1*b*
4	β-Mangostin	77[c]	2*b*
5	Garcinone E	30[c]	4
6	Egonol	5[c]	5
7	Garcinone-B	2[c]	7
8	6-Deoxy-γ-mangostin	2[c]	8
9	1,5,8-Trihydroxy-3-methoxy-2-(3-methyl-2-butenyl)xanthone	10[c]	1*a*
10	α-Mangostin	77[d]	2*a*
11	γ-Mangostin	20[d]	9

a) Dried powder 300 g. *b*) Elution order from SiO_2 column. *c*) The amount separated from a part (1.9 g) of the mother solution (residue, 4.5 g) of the benzene extract. *d*) The crystals were purified by a medium pressure column chromatography (MPLC) using 100 mg of the crude crystals (17 g) obtained by benzene extraction.

TABLE II. ^{1}H-NMR Assignments of Mangostin Derivatives

No.	γ-Mangostin (DMSO-d_6)	Garcinone E ($CDCl_3$)
4	6.33 (1H, s)	6.33 (1H, s)
5	6.75 (1H, s)	
12,22, (17)	5.19 (2H, m)	5.29 (3H, m)
11	4.04 (2H, d, J=6.6)	4.29 (2H, d, J=6.9)
21	3.22 (2H, d, J=6.6)	3.45 (2H, d, J=7.2)
16		3.60 (2H, d, J=7.4)
14, 15, 24, 25, (19, 20)	1.78—1.61 (CH_3 × 4, s)	1.88—1.71 (CH_3 × 6, s)
1-OH	13.89 (1H, s)	13.84 (1H, s)
OH × 3	11.16, 10.73, 8.64 (each s)	a)
1,3,6,7-Tetrahydroxy-2,8-bis-(3-methyl-2-butenyl)xanthone		1,3,6,7-Tetrahydroxy-2,5,8-tri-(3-methyl-2-butenyl)xanthone

a) In $CDCl_3$, the signals of the hydroxy groups did not appear.

TABLE III. ^{13}C-NMR Assignments of Mangostin Derivative

No.	γ-Mangostin (DMSO-d_6)	Garcinone E ($CDCl_3$)
1	159.76	160.51
2	109.25	108.18
3	161.94	161.51
4	91.98	93.16
4a	154.12	155.08
5	100.07	113.30
6	151.84[a]	148.72[a]
7	140.76	139.26
8	128.23	124.60
8a	109.96	111.30
9	181.51	183.05
9a	101.88	103.65
10a	152.38[a]	151.26[a]
11	25.26	25.83
12	123.68[b]	121.85[b]
13	130.23[c]	135.78[c]
14	25.56[d]	25.87[d]
15	18.00[e]	18.04[e]
16		22.56
17		121.02[b]
18		135.13[c]
19		25.83[d]
20		17.98[e]
21	20.91	21.45
22	122.52[b]	121.52[b]
23	129.97[c]	133.66[c]
24	25.41[d]	25.77[d]
25	17.61[e]	17.90[e]

a—e) Values bearing the same superscript within each column may be interchanged.

δ 3.45, 3.60 and 4.29 (each 2H, d), and three olefinic methines at 5.29 (3H, m), indicated the presence of an additional prenyl side chain, a 3-methyl-2-butenyl group, compared with γ-mangostin (**3**). In particular, the signal of a methylene group (d, 4.29) was deshielded due to an anisotropic effect of the carbonyl group, indicating that one of the 3-methyl-2-butenyl groups is located on C-8, as in γ-mangostin (**3**).

The ^{13}C-NMR spectrum of **1** indicated the presence of an additional 3-methyl-2-butenyl group on C-5 of γ-mangostin (**3**) as shown in Table II. All protonated carbons of **1** were assigned on the basis of ^{13}C–^{1}H correlation spectroscopy (COSY). The proton signal at δ 13.84 (s, 1H) of the hydroxy group on C-1 exhibited a strong hydrogen bonding with a carbonyl group on C-9. The aromatic proton and carbon signals at δ 6.33 (1H, s) and δ 93.16 in the NMR spectra of **1** showed a definite cross peak on ^{13}C–^{1}H COSY and they were readily assigned to H-4 and C-4 by comparison with the chemical shifts of known xanthones having no substituents on the C-4 position.[2] In a correlation *via* long-range coupling (COLOC) experiment, the signal of hydrogen on the C-1 hydroxy group was correlated to the C-1 and C-9a quaternary carbon signals, whereas the C-1 carbon signal showed a distinct cross peak with the doublet methylene signal on C-21 of the 3-methyl-2-butenyl side chain. Furthermore, an aromatic hydrogen signal on C-4 was observed to be coupled not only with the C-9a carbon, but also with the C-3 carbon bearing a hydroxy group. The C-3 carbon signal also had a long-range coupling with the above-mentioned methylene protons on C-21 of **1**. These connectivities in the COLOC experiment on **1** were also observed in the same measurement performed on the A-ring of γ-mangostin (**3**), as described in the experimental section. The doublet methylene signal at 4.29 due to C-11 in the 3-methyl-2-butenyl side chain was strongly deshielded by a carbonyl group at C-9, and showed cross peaks with the signals of C-7, C-8 and C-8a. The doublet methylene signal at 3.60 due to C-16 on another 3-methyl-2-butenyl side chain exhibited cross correlation with the signals of C-5, C-6 and C-10a. At this stage, the structure of garcinone E (**1**) was suggested to be 5-(3-methyl-2-butenyl)-γ-mangostin, as postulated previously.[4] Some supporting results were derived from the NMR spectral analysis of the methylated derivatives, **1a** and **1b**. The ^{1}H-NMR spectrum of **1a** showed the presence of a hydroxy group (1H, s, 13.44) chelated to the carbonyl group on C-9 and two methoxy signals at 3.92 and 3.90. In the UV spectrum, **1a** showed no position shift of absorption maxima upon addition of aqueous NaOAc, in contrast to β-mangostin (**4**), which showed a red shift on addition of aqueous NaOAc.[9] This indicated that the new methoxy groups were on C-3 and C-6, *para* to the xanthone carbonyl. The proton signal of a methoxy group (3H, s, δ 3.92) showed a distinct cross peak with the C-4 aromatic proton (1H, s, δ 6.34) in the ^{1}H–^{1}H nuclear Overhauser effect spectroscopy (NOESY) experiment. The ^{1}H-NMR spectra of **1b** showed a chelated hydroxy group as described previously and three methoxy protons at δ 3.96, 3.92 and 3.79. Similarly to the case of **1a**, a ^{1}H–^{1}H NOESY experiment on **1b** exhibited a cross peak between the C-3 methoxy group (3H, s, δ 3.92) and C-4 aromatic proton at δ 6.35 (s). This was further supported by the differential NOE technique; the signal areas of the protons at δ 3.92 and 6.35 increased by 11% and 16% upon irradiation of the other, respectively. All of the ^{1}H signals of **1b** gave reasonable cross peaks to the corresponding ^{13}C signals in a COLOC experiment, as described in the experimental section.

Thus, garcinone E (**1**) was established as 1,3,6,7-tetrahydroxy-2,5,8-tri-(3-methyl-2-butenyl)xanthene-9-one. Garcinone E might be a precursor of tovophyllin-A.[10]

The structures of other known compounds in Table I were assigned on the basis of UV, mass, ^{1}H- and ^{13}C-NMR and two dimensional (2D) NMR spectra as well as comparison with the data in the literature.

A non xanthone derivative, egonol, was isolated for the first time from the fruit hulls.

Experimental

All melting points were determined on a Yamato MP-21 apparatus and are uncorrected. The instruments used in this study were as follows: UV spectra, Hitachi U3400 spectrophotometer; infrared (IR) spectra, Hitachi 260 spectrophotometer; MS, Hitachi M-60 and RMU-7M spectrometers; ^{1}H-(500 MHz) and ^{13}C-(125 MHz)NMR spectra were taken with a JEOL JNM A 500 instrument in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as an internal standard. Thin layer chromatography was performed on Merck precoated Silica gel $60F_{254}$ plates. Column chromatography was carried out on Merck Silica gel 60 (230—4000 mesh for flash chromatography), pre-packed columns (silica gel, Kusano CPS-HS-221-05 for MPLC), and Merck Al_2O_3 90 (activity II—III). Abbreviations used are: singlet (s), doublet (d), triplet (t), multiplet (m), shoulder (sh).

Plant Material The fruit hulls of *G. mangostana* were purchased in Chiang Mai City, Thailand in 1990.

Garcinone E (1) Compound **1** was recrystallized from EtOAc–hexane as fine yellow crystals (30 mg), mp 152.5—155.5 °C; λ_{max}^{EtOH} nm (abs.), 367 (0.61), 322 (0.62), 260 (1.19), 244 (1.34), λ_{min}^{EtOH} nm (abs.), 340 (0.47), 289 (0.28), 254 (1.14), 224 (0.95), $\lambda_{max}^{EtOH-NaOAc}$ nm (abs.), 370 (0.83), 260 (1.09), 244 (1.27), $\lambda_{min}^{EtOH-NaOAc}$ nm (abs.), 299 (0.20), 256 (1.07), 223 (0.91). HR-EIMS *m/z*: 464.2203 ([M]$^+$, base peak), (Calcd for $C_{28}H_{32}O_6$: 464.2199). COLOC; (in $CDCl_3$; crossed peaks with ^{13}C-NMR signals, ppm), 3.47 (H-21; 108.18 C-2, 160.51 C-1, 161.51 C-3), 3.60 (H-16; 113.30 C-5, 148.72 C-6 (or 10a), 151.26 C10a (or 6)), 4.31 (H-11; 111.30 C-8a, 124.60 C-8, 139.26 C-7), 5.29 (H-12, 17, 22; no crossed peak), 6.33 (H-4; 103.65 C-9a, 155.08 C-4a, 161.51 C-3), 13.84 (C-1 OH; 103.65 C-9a, 160.51 C-1).

Methylation of Garcinone E (1) with CH_2N_2 An Et_2O solution of CH_2N_2 was added to an ice cooled methanol solution of **1** (15 mg) and the reaction mixture was purified by MPLC (3% EtOAc–hexane) to afford **1a** (4 mg, an amorphous powder) and **1b** (6 mg, an amorphous powder). **1a**: MS *m/z* (rel. int.): 492 ($C_{30}H_{36}O_6$, [M]$^+$ 93), 449 (77), 421 (62), 393 (100): ^{1}H-NMR ($CDCl_3$, ppm); 1.56, 1.68, 1.70, 1.71, 1.80, 1.87 (s, each $CH_3 \times 6$), 3.36 [2H, d, J=7.1, H-21. The signal was assigned on the basis of a comparison with the chemical shift of H-21 methylenes of known xanthones, γ-mangostin (**3**), 3.44, J=7.15; β-mangostin (**4**), 3.35, J=7.15 Hz and α-mangostin (**2**), 3.22, d, J=6.9 Hz], 3.60 (2H, d, J=6.9 Hz, H-16), 3.90 (3H, s, C-6 OCH_3), 3.92 (3H, s, C-3 OCH_3), 4.17 (2H, d, J=6.9 Hz, H-11), 5.23 (1H, dd, J=8.0 Hz, H-22), 5.24 (1H, dd, J=8.0 Hz, H-17), 5.30 (1H, dd, J=8.0 Hz, H-12), 5.67 (1H, s, C-7 OH), 6.34 (1H, s, arom. H-4), 13.44 (1H, s, C-1 OH). UV λ_{max}^{EtOH} nm (abs.): 376 (0.26), 314 (0.72), 267 (1.27), 245 (1.07), $\lambda_{max}^{EtOH-NaOAc}$ nm (abs.): 377 (0.23), 316 (0.63), 267 (1.11), 245 (0.94). **1b**: HR-EIMS, (rel. int.): 506.2671 ([M]$^+$ Calcd for $C_{31}H_{38}O_6$, 506.2668, 100), 435 (55), 381 (33). COLOC; (in $CDCl_3$; crossed peaks with ^{13}C-NMR signals, ppm), 3.36 (2H, d, J=7.3, H-21; 111.45 C-2, 159.80 C-1), 3.59 (2H, d, J=7.0, H-16; 121.91 C-5, 152.53 C-10a), 3.79 (3H, s, C-7 OCH_3; 147.87 C-7), 3.92 (3H, s, C-3 OCH_3; 163.64 C-3), 3.96 (3H, s, C-6 OCH_3; 156.34 C-6), 4.12 (2H, d, J=6.4, H-11; 115.06 C-8a, 123.64 C-12, 131.50 C-13, 135.47 C-8, 147.87 C-7), 5.24 (3H, m, H-12, H-17, H-22; no crossed peak), 6.35 (1H, s, arom. H-4; 103.97 C-9a, 111.45 C-2, 155.37 C-4a), 13.43 (1H, s, C-1 OH; 103.97 C-9a, 111.45 C-2, 159.80 C-1). The ^{1}H- and ^{13}C-NMR signals of two 3-methyl-2-butenyl side chains on C 2 and C 5 could not be distinguished. Thus, assignments of corresponding signals in the ^{1}H- or ^{13}C-NMR spectrum are interchangeable.

γ-Mangostin (3) COLOC (in DMSO-d_6; crossed peaks with ^{13}C-NMR signals, ppm); 3.22 (2H, d, J=6.9 Hz, H-21a, b; 109.25 C-2, 159.76 C-1, 161.94 C-3), 4.04 (2H, d, J=6.6 Hz, H-11a, b; no crossed peaks), 6.33 (1H, s, arom. H-4; 101.88 C-9a, 154.12 C-4a, 161.94 C-3), 6.75 (1H, s, arom. H-5; 109.96 C-8a, 140.76 C-7, 151.85 C-10a (or 6), 152.38 C-6 (or 10a).

Egonol This compound (an amorphous material) was isolated in such a small amount that crystallization could not be attempted. HR-EIMS: *m/z* (rel. int.) 326.1154 ([M]$^+$ Calcd for $C_{19}H_{18}O_5$, 326.1160, 78), 282 (100). UV λ_{max}^{EtOH} nm (abs.): 316 (1.24) 216 (1.53). All proton signals were assigned on the basis of a ^{1}H–^{1}H COSY experiment; the results were in accordance with a 1D ^{1}H-NMR assignment in the literature.[5]

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