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THREE XANTHONES FROM *GARCINIA SUBELLIPTICA*

HIROYUKI MINAMI, EMI TAKAHASHI, MITSUAKI KODAMA and YOSHIYASU FUKUYAMA*

Institute of Pharmacognosy, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

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Key Word Index—*Garcinia subelliptica*; Guttiferae; wood; 2,5-dihydroxy-1-methoxyxanthone; 1-*O*-methylsymphoxanthone; garciniaxanthone E; symphoxanthone; subelliptenone A; xanthone.

Abstract—Three new xanthones, 2,5-dihydroxy-1-methoxylxanthone, 1-*O*-methylsymphoxanthone and garciniaxanthone E, have been isolated along with the previously known symphoxanthone and subelliptenone A from the wood of *Garcinia subelliptica*. Their structures have been elucidated mainly on the basis of spectroscopic data and confirmed by converting them into the corresponding known compounds. Garciniaxanthone E is the second geranylated xanthone isolated from *Garcinia* species.

INTRODUCTION

Garcinia species are well known to be rich in a variety of oxygenated and prenylated xanthones [1]. Some of these exhibit a wide range of biological and pharmacological activities, e.g., cytotoxic [2], antiinflammatory [3, 4], antimicrobial [5] and antifungal [6] activity, as well as xanthine oxidase and monoamine oxidase inhibitory activities [7, 8]. In the course of our search for biologically active substances in the wood of *G. subelliptica*, we have isolated a number of new xanthones, including garciniaxanthones A and B [9] having interesting neurotrophic properties, as well as garciniaxanthones C [10] and D [11] showing antioxidant properties. Further investigation on the chemical constituents of the wood of the title species resulted in the isolation of three new xanthones **1**, **3** and **5**, along with symphoxanthone (**4**) [12] and subelliptenone A (**6**) already isolated from the root bark of *G. subelliptica* [13]. We report herein the isolation and structural determination of the three new compounds.

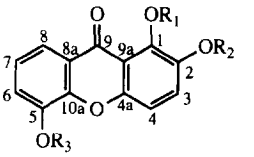
RESULTS AND DISCUSSION

The wood of *G. subelliptica* was extracted with ethyl acetate and the ethyl acetate extract fractionated by Celite column chromatography eluting with *n*-hexane, methylene chloride, ethyl acetate and methanol. The fraction eluted with ethyl acetate was fractionated by repeated silica gel and reverse-phase C_{18} column chromatography to afford 2,5-dihydroxy-1-methoxyxanthone (**1**), 1-*O*-methylsymphoxanthone (**3**), garciniaxanthone E (**5**), symphoxanthone (**4**) and subelliptenone A (**6**).

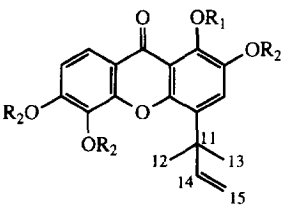
Compound **1**, obtained as orange needles, had a molecular formula of $C_{14}H_{10}O_5$ established by HREI mass spectrometry (*m/z* 258.0494 $[M]^+$). Its IR and UV spectra showed absorptions characteristic of a hydroxylated xanthone. The ^1H NMR spectrum (Table 1) of **1** contained a methoxyl signal at δ_H 3.80, *ortho*-coupled aromatic signals at δ_H 7.29 and 7.38 (each *d*, $J = 9.3$ Hz) and an ABM-system signal at δ_H 7.19 (*t*, $J = 7.8$ Hz), 7.24 (*dd*, $J = 7.8$, 1.5 Hz) and 7.53 (*dd*, $J = 7.8$, 1.5 Hz) assignable to a 1,2,3-trisubstituted benzene ring, in addition to two hydroxyl signals at δ_H 10.31 and 9.48. These spectral data implied that one of the three hydroxyl groups on 1,3,5-trihydroxyxanthone (**2**), which was previously reported as an antioxidative xanthone [10], should be methylated. The methylated derivative **1a** was found to be identical in all respects with the compound transformed from **2** with MeI-NaH in DMF. The sole methoxyl group in **1** was shown to be located at the C-1 position on the basis of the following evidence. Irradiation of the methoxyl signal caused no NOE interaction against the other aromatic proton signals and the C-9 carbonyl signal apeared at a higher field (175.4 ppm) (Table 2) than normal value (~ 180 ppm) due to the steric effect of the bulky methoxyl group [14]. Thus, **1** was identified as 2,5-dihydroxy-1-methoxyxanthone.

Compound **3** had a molecular formula of $C_{19}H_{18}O_6$ ($[M]^+$ at *m/z* 342.1075). Its spectral data also disclosed that it was xanthone (UV 235 and 258 nm; IR 1642, 1607 and 1472 cm^{-1}) bearing one methoxyl group (δ_H 3.77), three hydroxyl groups (IR 3335 cm^{-1}; δ_H 8.30, 9.0 and 9.25) and a 1,1-dimethyl-2-propenyl group [δ_H 1.60 (6H, *s*), 5.09 (1H, *d*, $J = 17.6$ Hz), 5.09 (1H, *d*, $J = 11.0$ Hz) and

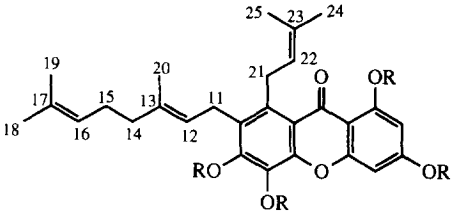
*Author to whom correspondence should be addressed.



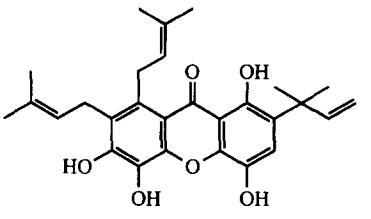
	R_1	R_2	R_3
1	Me	H	H
1a	Me	Me	Me
2	H	H	H



	R_1	R_2
3	Me	H
3a	Me	Me
4	H	H



	R
5	H
5a	Me



6

Table 1. ^{1}H NMR spectral data of compounds **1**, **1a**, **3**, and **3a**

H	1*	1a†	3*	3a†
3	7.38 *d* (9.3)§	7.37 *d* (8.0)	7.27 *s*	7.26 *s*
4	7.29 *d* (9.3)	7.35 *d* (8.0)	—	—
6	7.24 *dd* (7.8, 1.5)	7.26 *dd* (7.8, 1.9)	—	—
7	7.19 *t* (7.8)	7.19 *t* (7.8)	6.89 *d* (8.8)	6.97 *d* (9.0)
8	7.53 *dd* (7.8, 1.5)	7.87 *dd* (7.8, 1.9)	7.45 *d* (8.8)	8.03 *d* (9.0)
12	—	—	1.60 *s*	1.68 *s*
13	—	—	1.60 *s*	1.68 *s*
14	—	—	6.39 *dd* (17.6, 11.0)	6.35 *dd* (16.0, 10.4)
15	—	—	5.09 *d* (17.6)	5.09 *d* (16.0)
			5.09 *d* (11.0)	5.09 *d* (10.4)
C_1-OH	—	—	—	—
C_2-OH	9.48 *s*	—	8.30 *s*	—
C_5-OH	10.31 *s*	—	9.00 *s*	—
C_6-OH	—	—	9.25 *s*	—
C_1-OMe	3.80 *s*	4.02 *s*	3.77 *s*	4.00 *s*
C_2-OMe	—	3.94 *s*	—	3.94 *s*
C_5-OMe	—	4.03 *s*	—	3.97 *s*
C_6-OMe	—	—	—	3.99 *s*

*In DMSO-d_6 on 400 MHz.
†In $CDCl_3$ on 200 MHz.
§Coupling constants (*J* in Hz) are given in parentheses.

6.39 (1H, *dd*, *J* = 17.6 and 11.0 Hz)]. Additionally, the ^{1}H NMR of **3** contained *ortho*-coupled aromatic signals at δ_H 6.89 (*d*, *J* = 8.8 Hz) and 7.45 (*d*, *J* = 8.8 Hz) and a singlet aromatic signal at δ_H 7.27, which showed NOEs to the methyl signal at δ_H 1.60. The above spectral data suggested that **3** was closely related to symphoxanthone (**4**) [12] except for the presence of the extra methoxyl group. This was substantiated by identification of the methyl derivative **3a** with the tetramethylated compound derived from **4** by the same procedure used for the preparation of **1a**. The remaining question was which hydroxyl group in **4** is methylated. Upon irradiation of the methoxyl signal in **3**, no NOE was observed. Also, the chemical shift value for the C-9 carbonyl carbon was

Table 2. ^{13}C NMR spectral data of compounds **1** and **3** (100 MHz, in DMSO-d_6)

C	1	3
1	145.1	143.4
2	146.6	145.3
3	124.0	121.2
4	113.7	132.3
4a	149.5	147.9
5	146.2	132.3
6	119.3	145.3
7	123.4	112.5
8	115.2	115.2
8a	122.6	116.2
9	175.4	175.3
9a	116.1	115.9
10a	144.4	150.3
11	—	40.3
12	—	26.8
13	—	26.8
14	—	146.7
15	—	111.4
C_1-OMe	61.0	60.7

175.3 ppm (Table 2) in comparison with that (183 ppm) of **4**. As in the case of **1**, the structure of **3** was thus assigned as 1-*O*-methylsymphoxanthone.

Compound **5**, obtained as orange gummy oil, had a molecular formula of $C_{28}H_{32}O_6$ ($[M]^+$ at *m/z* 464.2197). Its UV and IR displayed absorptions characteristic of a substituted xanthone. The ^1H NMR spectrum of **5** showed the presence of four hydroxyl groups with a chelated one (δ_H 13.56) and *meta*-coupled aromatic protons resonating at δ_H 6.11 (*d*, J = 1.5 Hz) and 6.36 (*d*, J = 1.5 Hz). The presence of the four hydroxyl groups was confirmed unambiguously by converting **5** into the tetramethoxyl derivative **5a** with MeI-NaH in DMF. In the NOE experiment of **5a**, the sole *meta*-coupled aromatic protons resonated at δ_H 6.32 (*d*, J = 2.4 Hz) and 6.51 (*d*, J = 2.4 Hz) caused NOE interaction with the methoxyl signal at δ_H 3.90; thereby, **5** had a 1,3-hydroxylated benzene nucleus as the right hand xanthone ring. From additional NMR data (Table 3) assisted by DQFCOSY and HMQC, there were five olefinic methyl groups [δ_H 1.52, 1.58, 1.62 and 1.70 (each 3H); δ_C 25.4, 17.5, 25.4, 16.1 and 17.9] which made up three trisubstituted double bonds [δ_H 5.03 (*t*, J = 6.8 Hz), 4.97 (*t*, J = 5.9 Hz) and 4.99 (1H, *m*)], which further extended to connect to four methylenes [δ_H 1.92 (2H, *t*, J = 7.3 Hz), 2.01 (2H, *dt*, J = 7.3, 6.8 Hz), 3.31 (2H, *d*, J = 5.9 Hz) and 3.96 (2H, *br s*)], suggesting the presence of 3-methyl-2-butenyl and geranyl groups. These spectral data also indicated that the left hand xanthone ring must be fully substituted with the two hydroxyl, 3-methyl-2-butenyl and geranyl groups. The chemical shift value (δ_H 3.96) of C-21 on the 3-methyl-2-butenyl group resonated at a lower field than that (δ_H 3.39) of C-11 on the geranyl group due to a magnetic deshielding effect by the C-9 carbonyl carbon [13, 15], accounting for the location of

Table 3. ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compounds **5** (in DMSO-d_6) and **5a** (in $CDCl_3$)

	5		5a
Position	^1H	^{13}C	^1H
1	—	163.2	—
2	6.11 *d* (1.5)*	97.7	6.32 *d* (2.4)
3	—	164.5	—
4	6.36 *d* (1.5)	93.0	6.51 *d* (2.4)
4a	—	156.1	—
5	—	129.9	—
6	—	149.9	—
7	—	125.2	—
8	—	132.9	—
8a	—	110.1	—
9	—	181.9	—
9a	—	102.1	—
10a	—	145.7	—
11	3.31 *d* (5.9)	24.1	3.39 *d* (6.4)
12	4.97 *t* (5.9)	122.8	5.03 *t* (6.4)
13	—	134.3	—
14	1.92 *t* (7.3)	39.3	1.99 *m*
15	2.01 *td* (7.3, 6.8)	26.0	2.07 *m*
16	5.03 *t* (6.8)	124.0	5.06 *t* (6.8)
17	—	130.7	—
18	1.52 *s*	25.4	1.56 *s*
19	1.58 *s*	17.5	1.64 *s*
20	1.70 *s*	16.1	1.77 *s*
21	3.96 *m*	27.9	3.99 *m*
22	4.99 *m*	124.4	5.20 *m*
23	—	129.5	—
24	1.62 *s*	25.4	1.67 *s*
25	1.70 *s*	17.9	1.74 *s*
C_1-OH	13.56 *s*	—	—
C_3-OH	10.81 *s*	—	—
C_5-OH	9.52 *s*	—	—
C_6-OH	9.88 *s*	—	—
C_1-OMe	—	—	3.96 *s*
C_3-OMe	—	—	3.90 *s*
C_5-OMe	—	—	3.95 *s*
C_6-OMe	—	—	3.99 *s*

*Coupling constants (J in Hz) are given in parentheses.

the 3-methyl-2-butenyl group at the C-8 position. This was substantiated by HMBC correlation of the H-21 signal to the C-8a carbon signal at δ_C 110.1 (Table 4). Furthermore, the proton signal due to the benzylic H-11 on the geranyl part showed cross-peaks with the C-6 (δ_C 149.9), C-7 (δ_C 125.2) and C-8 (δ_C 132.9) signals, amongst which the C-7 and C-8 shared HMBC correlations with the H-21, thereby confirming the geranyl group at the C-7 position. The left hand xanthone ring, therefore, was substituted in turn with the two hydroxyl groups, geranyl and the 3-methyl-2-butenyl groups at the C-5, 6, 7 and 8 positions, respectively. Accordingly, the structure of garciniaxanthone E (**5**) was assigned as 7-geranyl-1,3,5,6-tetrahydroxy-8-(3-methyl-2-butenyl)xanthone.

All the xanthones isolated in the present study exhibited no antioxidant activity at a concentration as high as 10 $\mu g ml^{-1}$ [10, 16–18]. It should be noted that

Table 4. ^{13}C-^{1}H Correlation on the xanthone ring of **5** through three bonds or two bonds in the HMBC experiment*

C	Correlated proton
1	C_1-OH
2	C_1-OH, C_3-OH, H-4
3	C_3-OH
4	H-2, C_3-OH
4a	—
5	C_5-OH, C_6-OH
6	H-11, C_5-OH, C_6-OH
7	H-11, H-21, C_6-OH
8	H-11, H-21
8a	H-21
9	—
9a	H-2, H-4, C_1-OH
10a	C_5-OH

*$J_{C\text{-}H}$ = 8.1 Hz

garciniaxanthone E (**5**) is the second example of a geranylated xanthone isolated from *Garcinia* species [1, 15, 19].

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR: TMS as int. standard. CC: silica gel (Merck, 230–400 mesh and Wakogel C-300) and Sephadex LH-20 (25–100 μm, Pharmacia). TLC: precoated silica gel F_{254} (Merck); spots were visualized by UV (254 nm) and 10% $CeSO_4$–H_2SO_4.

Plant material. Wood of *G. subelliptica* Merr. collected from Ishigaki Island, Japan, was identified by Dr H. Murata (Ibushuki, Kagoshima, Japan).

Extraction and isolation. The EtOAc extract (150 g) was mixed with Celite (150 g) and the solvent removed *in vacuo* to give solids, which were pulverized. The resultant powder was packed into a glass column and then eluted in turn with *n*-hexane (1.5 l), CH_2Cl_2 (1.5 l), EtOAc (1 l) and MeOH (1 l) giving 6 frs (1–6). Fr. 3 (125 g) was chromatographed by CC on silica gel with CH_2Cl_2–EtOAc (1:3) to give 11 frs (7–18). Fr. 10 (2.2 g) was again chromatographed by CC on Cosmosil 75C_{18}-OPN with MeOH–MeCN–H_2O (1:1:2.5) followed by HPLC [Cosmosil 5C_{18}-AR (ϕ 10 × 250 mm), MeOH–MeCN–H_2O (1:1:3; 2 ml min^{-1})] to afford 2,5-dihydroxy-1-methoxyxanthone (**1**) (20 mg). Fr. 11 (203 mg) was purified by repeated CC on silica gel (C-300) with CH_2Cl_2–EtOAc (3:1) to give 1-*O*-methylsymphoxanthone (**2**) (27 mg) and symphoxanthone (**4**) (50 mg). Fr. 9 (5 g) was chromatographed by CC on silica gel (C-300) with CH_2Cl_2–EtOAc (1:2) followed by reverse-phase on Cosmosil 75C_{18}-OPN with MeOH–H_2O (8:1) to give garciniaxanthone E (**5**) (12 mg) and subelliptenone A (**6**) (10 mg).

2,5-Dihydroxy-1-methoxyxanthone (**1**). Orange prisms, mp 214–218°. EIMS *m/z* (rel. int.): 258.0494 $[M]^+$ (calc. 258.0528 for $C_{14}H_{10}O_5$) (76), 240 $[M-18]^+$ (100). UV λ_{max}^{EtOH} nm (ε): 203 (26300), 242 (56800), 256 (56800). IR ν_{max}^{film} cm^{-1}: 3150 (OH), 1635 (C=O), 1595 and 1495 (aroma,). ^{1}H and ^{13}C NMR: Tables 1 and 2.

Methylation of **1**. To an ice-cooled soln of **1** (5 mg) in DMF (1 ml) was added NaH (3.7 mg) and stirring continued for 10 min. After MeI (0.1 ml) was added, the reaction mixt. was stirred at room temp. for 24 hr. The reaction mixt. was then dild with H_2O and extracted with Et_2O. The extracts were washed with H_2O, satd. $NaHCO_3$ and satd. NaCl solns. After drying ($MgSO_4$), solvent was evapd *in vacuo* and the residue chromatographed on silica gel (CH_2Cl_2–EtOAc, 3:2) to yield **1a** (4.1 mg) as an amorphous powder. EIMS *m/z* (rel. intl.): 286.0838 $[M]^+$ (94) (calc. 286.0841 for $C_{16}H_{14}O_5$), 271 $[M-15]^+$ (100). ^{1}H NMR: Table 1.

1-O-Methylsymphoxanthone (**3**). Orange amorphous. EIMS *m/z* (rel. int.): 342.1075 $[M]^+$ (100) (calc. 342.1103 for $C_{19}H_{18}O_6$), 324 $[M-18]^+$ (79), 309 (48), 295 (45), 281 (63), 267 (33). UV λ_{max}^{EtOH} nm (ε): 202 (31300), 235 (44700), 258 (55700). IR ν_{max}^{film} cm^{-1}: 3335 (OH), 1642 (C=O), 1607 and 1472 (arom.). ^{1}H and ^{13}C NMR: Tables 1 and 2.

Methylation of **3**. The tetramethoxyl derivative **3a** (4 mg) was derived from **3** (5 mg) in a similar manner to that described for the prepn of **1a**. IR ν_{max}^{film} cm^{-1}: 1657 (C=O), 1610 (C=CH_2), 1589 and 1462 (arom.). EIMS *m/z* (rel. int.): 384.1584 $[M]^+$ (100) (calc. 384.1573 for $C_{22}H_{24}O_6$), 369 $[M-15]^+$ (92), 355 (50). ^{1}H NMR: Table 1.

Garciniaxanthone E (**5**). Orange gummy oil. EIMS *m/z* (rel. int.): 464.2197 $[M]^+$ (36) (calc. 464.2199 for $C_{28}H_{32}O_6$), 395 $[M-69]^+$ (24), 339 $[M-125]^+$ (84), 325 (66). UV λ_{max}^{EtOH} nm (ε), 207 (27900), 252 (34000), 327 (9100). IR ν_{max}^{KBr} cm^{-1}: 3508 (OH), 1660 (C=O), 1602 and 1566 (arom.). ^{1}H and ^{13}C NMR: Table 3.

Methylation of **5**. The tetramethoxyl derivative **5a** (2.4 mg) was derived from **5** (5.6 mg) in a similar manner to that described for the prepn of **1a**. EIMS *m/z* (rel. int.) 520.2844 $[M]^+$ (6) (520.2825 for $C_{32}H_{40}O_6$), 451 $[M-69]^+$ (100), 395 $[M-125]^+$ (95), 355 (78). IR ν_{max}^{KBr} cm^{-1}: 1655 (C=O), 1610 and 1570 (arom.). ^{1}H NMR: Table 3.

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