NOVEL XANTHONES WITH SUPEROXIDE SCAVENGING ACTIVITY FROM *GARCINIA SUBELLIPTICA*

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Two new xanthones, garciniaxanthone D (**1**) containing a dihydrobenzofuran ring and 1,4,5-trihydroxyxanthone (**2**), have been isolated from *Garcinia subelliptica* as superoxide anion scavengers. Their structures have been determined mainly by spectroscopic methods and some chemical reactions.

KEY WORDS *Garcinia subelliptica*; garciniaxanthone D; 1,4,5-trihydroxyxanthone; antioxidant; anti lipid peroxidation; superoxide anion scavenger

Overgeneration of reactive superoxide radicals in the biological system has been known to induce lipid peroxidation and damage membranes, thus resulting in the initiation and/or the progression of a number of diseases.[1] Therefore, it is valuable to search for natural products which can protect membranes against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. We have already reported that the methanol extract of the woods of *G. subelliptica* exhibited antioxidant activity in the *in vitro* three assay sytems.[2] Continuing fractionation of this methanol extract monitored by bioassay led to the isolation of two new xanthones, garciniaxanthone D (**1**) and 1,4,5-trihydroxyxanthone (**2**), as active principles. In this communication, we report on the structural determination of two new compounds and on their superoxide scavenging activities.

The UV and IR spectra of garciniaxanthone D (**1**),[3] $C_{23}H_{24}O_7$, showed absorptions characteristic of a substituted xanthone. The presence of four hydroxyl groups was supported by the IR (3375 cm^{-1}) and the ^1H NMR (δ_H 12.91, 9.63, 6.05, and 4.78), followed by converting **1** to the trimethoxy derivative **1a** (*m/z* 454) with MeI-NaH in DMF. The intact OH on methylation must be tertiary. The substitution pattern on the right-hand benzene ring of **1** was conceived to be identical with that in a previously reported garciniaxanthone B (**3**),[4] since the ^1H and ^{13}C NMR data which were allocated to these regions in **1** were very similar to those in **3**. This was substantiated by HMBC and NOE experiments, as shown in Figs. 1 and 2. Taking a look at the left-hand benzene ring of the xanthone, the NMR data (Table I) of **1** showed the presence of *ortho* coupled aromatic protons at δ_H 7.40 (*d*, J =7.8 Hz) and 7.72 (*d*, J =7.8 Hz) assignable to H-7 and H-8, two O-bearing vicinal methines [δ_H 5.41 (*dd*, J = 7.3, 4.9 Hz) and δ_C 71.8; δ_H 4.38 (*d*, J = 4.9 Hz) and δ_C 98.2], and two singlet methyl groups (δ_H 1.22 and 1.25;δ_C 25.5 and 25.8), which were correlated to an oxygen bearing quaternary carbon resonance at δ_C 69.7 in HMBC, thus indicating the presence of a dimethylcarbinol group. In additional HMBC, these two methyl signals correlated to the one

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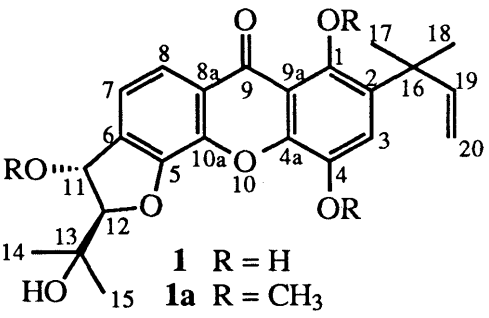

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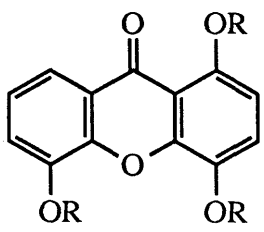
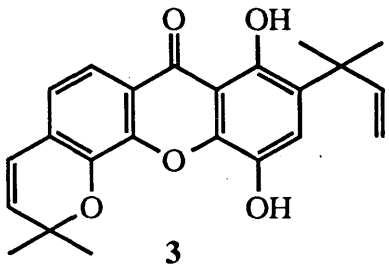
1a R = CH_3



2 R = H
2a R = CH_3



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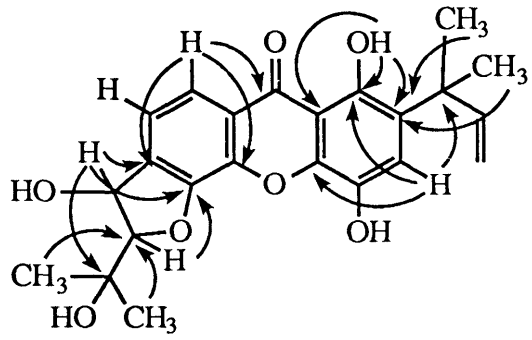

Fig 1. HMBC Correlations of Garciniaxanthone D (**1**)

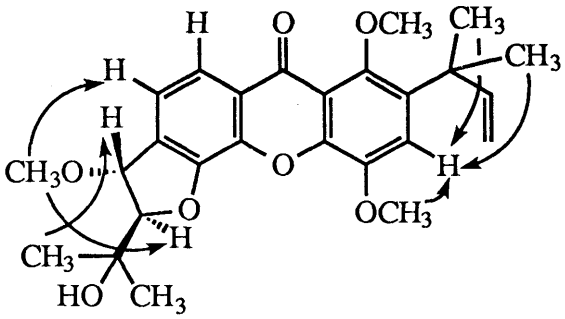

Fig 2. 2D NOESY Correlations of **1a**

(C-12) of the vicinal methine carbons, and thereby the dimethylcarbinol group must be connected to C-12. Furthermore, the methine proton signals at δ_H 5.41 (H-11) and at δ_H 4.38 (H-12) showed cross peaks with C-5 and C-6, and C-5, respectively, as shown in Fig.1, thus leading to the formation of a dihydrobenzofuran ring fused at the 5 and 6 positions. The relative stereochemistry of the substituents on the dihydrobenzofuran ring was elucidated based on 2D NOESY and difference NOE experiments of the trimethoxy derivative **1a**, as shown in Fig. 2. Consequently, OCH_3 at the 11 position and the dimethylcarbinol group at the 12 position take a *trans*-relationship to each other. Thus, the structure of garciniaxanthone D was determined as **1**.

Compound **2**,[5] $C_{13}H_8O_5$, was also regarded as a substituted xanthone by its UV and IR spectra. The IR and ^1H NMR spectrum of **2** indicated the presence of three hydroxyl groups containing a chelated one (3400~2700 cm^{-1}; δ_H 11.82, 10.20 and 9.41). In fact, methylation of **2** with MeI-NaH in DMF yielded the trimethoxy derivative **2a** (*m/z* 286). The ^1H NMR of **2** contained *ortho* coupled aromatic signals at δ_H 6.67 (*d*, *J* = 8.8 Hz) and 7.28 (*d*, *J* = 8.8 Hz), and an ABM system signal at δ_H 7.30 (*t*, *J* = 7.8 Hz), 7.36 (*dd*, *J* = 7.8, 1.5 Hz) and 7.59 (*dd*, *J* = 7.8, 1.5 Hz) indicative of a 1, 2, 3-trisubstituted benzene ring, thus suggesting the locations of three hydroxyl groups at the 1, 4 and 5 positions. This was supported by the following NOE experiments: the selective irradiation of three methoxy proton signals at δ_H 3.98, 4.01, and 4.04 in **2a** caused NOE enhancements for H-2 at δ_H 6.73 (*d*, *J* = 9.0 Hz), H-3 at δ_H 7.19 (*d*, *J* = 9.0 Hz), and H-6 at δ_H 7.20 (*dd*, *J* = 8.1, 1.5 Hz), respectively. Accordingly, the structure of **2** was determined to be 1, 4, 5- trihydroxyxanthone.

Antioxidant properties for compounds **1** and **2** are summarized in Table II. In particular, **2** could scavenge approximately 90% of superoxide anion in xanthine and xanthine oxidase system at

a concentration as low as 5 μgml^{-1}.

Table I. ^{13}C NMR Spectral Data of **1**, **2**, **3** (in DMSO-d_6)

C	1	2	3	C	1	2	3
1	151.2	152.4	153.0	10a	141.1	144.7	144.9
2	127.4	109.1	126.6	11	71.8		121.8
3	122.6	123.3	122.7	12	98.2		133.8
4	136.3	137.4	135.1	13	69.7		78.3
4a	141.8	143.0	140.4	14	25.5		27.8
5	147.4	146.3	141.1	15	25.8		27.8
6	137.8	120.9	129.0	16	40.0		40.3
7	120.7	124.5	121.4	17	26.3		26.7
8	116.9	114.8	117.6	18	26.3		26.7
8a	120.5	120.7	121.0	19	146.6		147.0
9	182.5	182.1	182.2	20	110.8		110.6
9a	108.6	108.4	108.5				

Table II. Antioxidant Activity of **1** and **2**

	Conc (μgml^{-1})	1	2
ALP (%)[6]	10	80.4	64.6
	5	15.8	25.2
DPPH (%)[7]	10	93.7	73.3
	1	-	5.7
O_2^- (%)[8]	15	100.0	96.6
	5	44.4	90.0

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3) **1**: $[\alpha]_D^{22.5}$ -14.4 (c 2.3, EtOH); EIMS *m/z* (rel. int.): 412 (M^+, 85), 397 (100); HR-EIMS : 412.1510 (Calcd 412.1522 for $C_{23}H_{24}O_7$); IR (FT) cm^{-1}: 3375 (OH), 1635 (C=O), 1589, 1488 (aroma.), 1458 (C=C); UV λmax (EtOH) nm (ε): 237 (43600), 243 (49400), 256 (56100), 266 (44700), 320 (22200); ^{1}H-NMR (400MHz, DMSO-d_6) δ: 1.22 (3H, *s*, 14-CH_3), 1.25 (3H, *s*, 15-CH_3), 1.48 (6H, *s*, 17, 18-CH_3), 4.38 (1H, *d*, *J* = 4.9 Hz, H-12), 4.78 (1H, *s*, 13-OH), 5.01 (1H, *d*, *J* =17.6 Hz, H-20), 5.01 (1H, *d*, *J* =10.3 Hz, H-20), 5.41 (1H, *dd*, *J* = 7.3, 4.9 Hz, H-11), 6.05 (1H, *d*, *J* = 7.3 Hz, 11-OH), 6.24 (1H, *dd*, *J* = 10.3, 17.5 Hz, H-19), 7.36 (1H, *s*, H-3), 7.40 (1H, *d*, *J* = 7.8 Hz, H-7), 7.72 (1H, *d*, *J* = 7.8 Hz, H-8), 9.63 (1H, *s*, 4-OH), 12.91 (1H, *s*, 1-OH).

4) Y. Fukuyama, A. Kamiyama, Y. Mima, M. Kodama, *Phytochemistry*, **30**, 3433 (1991). Compound **3** had no antioxidant property in these assay systems.

5) **2**: EIMS *m/z* (rel. int. %): 244 (M^+, 100); HR-EIMS: 244.0350 (Cacld 244.0372 for $C_{13}H_8O_5$); IR (FT) cm^{-1}: 3400~2700 (OH), 1650 (C=O), 1599, 1502 (aroma.); UV λmax (EtOH) nm (ε): 246 (59600), 260 (42900), 319 (15500); ^{1}H-NMR (400MHz, DMSO-d_6) δ: 6.67 (1H, *d*, *J* = 8.8 Hz, H-2), 7.28 (1H, *d*, *J* = 8.8 Hz, H-3), 7.30 (1H, *t*, *J* = 7.8 Hz, H-7), 7.36 (1H, *dd*, *J* = 1.5, 7.8 Hz, H-6), 7.59 (1H, *dd*, *J* = 1.5, 7.8 Hz, H-8), 9.41 (1H, *s*, 4-OH), 10.20 (1H, *s*, 5-OH), 11.82 (1H, *s*, 1-OH).

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8) Superoxide anion scavenging activity (per cent inhibition in xanthine and xanthine oxidase system): J. M. McCord, I. Fridovich, *J. Biol. Chem.*, 244, 6049 (1969).

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