CONSTITUENTS OF THE SEEDS OF *GARCINIA KOLA*: TWO NEW ANTIOXIDANTS, GARCINOIC ACID AND GARCINAL[1]

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Abstract —— Two new chromanols, named garcinoic acid and garcinal, were isolated together with a known chromanol, δ-tocotrienol and three known biflavonoids, garcinianin, GB-1 and GB-2, from the seeds of *Garcinia kola* Heckel (Guttiferae) collected in Nigeria and their structures were characterized on the basis of the spectroscopic and chemical evidence. Garcinoic acid, garcinal and δ-tocotrienol showed about 1.5 times stronger antioxidation activity than that of *dl*-α-tocopherol.

Recently, we reported the isolation of two novel arylbenzofurans, a novel arylbenzopyran and a novel C-3/C-8"-flavanone/flavonol biflavonoid from the roots of *Garcinia kola* Heckel (Guttiferae)[2-4] collected in Nigeria. Our continuous study on the constituents of the above plant led to the isolation of two new chromanol derivatives, named garcinoic acid (**1**) and garcinal (**2**), together with a known chromanol, δ-tocotrienol (**3**), and a known biflavonoid, garcinianin (**4**), from the seeds, all of which are racemates. In this paper, we describe the isolation, structures and antioxidant activities of the constituents (**1** - **4**) of the seeds of *G. kola*.

Isolation The methanol extract of the seeds of *Garcinia kola* collected in Nigeria was separated by medium-pressure column chromatography (MPCC) on silica gel using a mixture of chloroform and methanol to six fractions. The first less-polar fraction was further separated by a combination of column chromatography and preparative thin layer chromatography to afford δ-tocotrienol (**3**), garcinal (**2**) and garcinoic acid (**1**) in 2.86, 0.035 and 0.031% yields, respectively. The third and fourth fractions contained the known biflavonoid, garcinianin (**4**), GB-1 (**5**) and GB-2 (**6**), which were identified by comparison of

the spectral data with the reported ones.[4,5] Although GB-1 (**5**) and GB-2 (**6**) were already isolated from the seeds of *G. kola* by Cotterill,[5] two known compounds, δ-tocotrienol (**3**) and garcinianin (**4**) were first isolated as the constituents of the seeds of the plant.

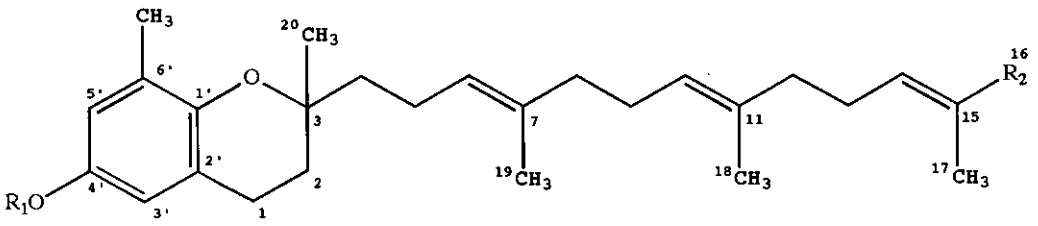
Structures Garcinoic acid (**1**) showed a molecular ion peak at *m/z* 426.2746 [M$^+$] (*m/z* 426.2767 Calcd for $C_{27}H_{38}O_4$) in the high-resolution EIMS spectrum. The presence of a chromanol chromophore was suggested by the UV[6,7] and EIMS[7] spectra (λ 296 nm (ε= 3800); *m/z* 426 (34), 177 (46), 137 (100)). This was further supported by the ^{1}H NMR (δ 1.24 (3H, s), 1.74 (2H, t, *J*=6.8 Hz), 2.10 (3H, s), 2.67 (2H, t, *J*=6.8 Hz), 6.36 (1H, d, *J*=2.9 Hz), 6.46 (1H, d, *J*=2.9 Hz) and ^{13}C NMR spectra (δ 16.0 (C-7'), 22.6 (C-1), 24.0 (C-20), 31.3 (C-2), 75.3 (C-3), 112.6 (C-3'), 115.7 (C-5'), 121.2 (C-2'), 127.3 (C-6'), 145.9 (C-1'), 147.7 (C-4')) of **1**. The chemical shift values corresponding to the chromanol of **1** are very similar to those of cystseirol A (**7**) from a marine source (δ 16.1 (C-7'), 22.6 (C-1), 24.9 (C-20), 31.1 (C-2), 75.3 (C-3), 112.5 (C-3'), 115.6 (C-5'), 121.2 (C-2'), 127.4 (C-6'), 146.3 (C-1'), 152.6 (C-4')).[8] The presence of three sets of a partial structure -CH_2-CH_2-CH=C(CH_3)- was shown by the ^{1}H NMR spectrum of **1** (δ 1.57 (2H, t, *J*=7.3 Hz), 2.09 (2H, dt, *J*=7.3, 7.6 Hz), 5.11 (1H, qt, *J*=1.5, 7.6 Hz), 1.57 (3H, d, *J*=1.5 Hz), 1.96 (2H, t, *J*=7.4 Hz), 2.05 (2H, dt, *J*=7.6, 7.4 Hz), 5.12 (1H, qt, *J*=1.5, 7.6 Hz), 1.58 (3H, d, *J*=1.5 Hz), 2.07 (2H, t, *J*=7.3 Hz), 2.26 (2H, dt, *J*=7.3, 7.7 Hz), 6.83 (1H, qt, *J*=1.5, 7.7 Hz), 1.81 (3H, d, *J*=1.5 Hz). The presence of an αβ-unsaturated carboxylic acid group was shown by the IR spectrum (ν 3400 br, 1690, 1648 cm^{-1}). The positions and stereochemistries of the methyl and carboxylic acid groups were determined as follows. The chemical shift values of three vinyl methyl groups in the ^{13}C NMR spectrum (δ 12.0, 15.8, 15.9) suggested the stereochemistries between the methyl groups and the corresponding vinyl protons to be all *trans*. From the above evidence, the structure of garcinoic acid should be represented as **1**. Recently, Davyt *et al.* reported the isolation of a new chromanol corresponding to 1,2-dehydrogarcinoic acid as its methyl ester after treatment with diazomethane from a marine source.[9]

Garcinal (**2**) showed a molecular ion peak at *m/z* 410.2809 [M$^+$] (*m/z* 410.2818 Calcd for $C_{27}H_{38}O_3$) in the high-resolution EIMS spectrum. The spectra of **2** are very similar to those of garcinoic acid (**1**) except for the signals due to an αβ-unsaturated aldehyde (λ 295 nm (ε 3300), ν 3400 br, 1684, 1647 cm^{-1}, δ_H 9.37 (1H, s), δ_C 195.3 (d)). The difference NOE experiment (15.5% enhancement from the vinyl proton to the formyl proton) showed the stereochemistry of the formyl group to be *E*-configuration. The structure was confirmed by the chemical transformation of garcinoic acid (**1**) to garcinal (**2**). **1** was converted to the methyl ester (**8**) by diazomethane at -78 °C, the methoxymethyl (MOM) ether (**9**) by MOM chloride, the primary alcohol (**10**) by lithium aluminum hydride, the aldehyde (**11**) by pyridinium dichromate (PDC), and then the phenol by hydrochloric acid, successively. The phenol was completely identical with garcinal (**2**). The third compound (**3**) showed an ion peak at *m/z* 396 [M$^+$], $C_{27}H_{40}O_2$. The spectra of **3** are very similar to those of garcinal (**2**) except for the signal due to a methyl group instead of a carbonyl group (λ 297 nm (ε 3300), ν 3400 br cm^{-1}, δ_H 1.64 (3H, s), δ_C 25.7 (q)). These data suggested **3** to be δ-

Table 1. ^{1}H and ^{13}C NMR Data of Garcinoic acid (**1**), Garcinal (**2**) and δ-Tocotrienol (**3**)[a,b]

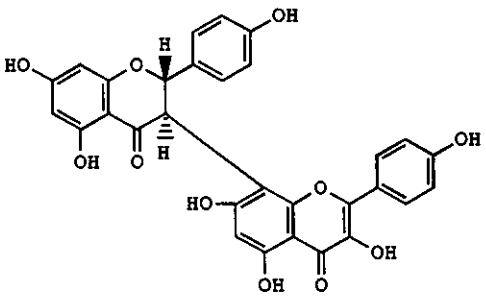
	Garcinoic acid (**1**)		Garcinal (**2**)		δ-Tocotrienol (**3**)	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	22.4 (t)	2.67 (t, 6.8)	22.5 (t)	2.70 (t, 7.0)	22.2 (t)	2.63 (t, 6.9)
2	31.3 (t)	1.74 (t, 6.8)	31.4 (t)	1.75 (t, 7.0)	31.3 (t)	1.68 (t, 6.9)
3	75.3 (s)		75.3 (s)		75.3 (s)	
4	39.5 (t)	1.57 (t, 7.3)	39.5 (t)	1.58 (t, 7.3)	39.7 (t)	1.53 (t, 7.3)
5	22.1 (t)	2.09 (dt, 7.3, 7.6)	22.2 (t)	2.13 (dt, 7.3, 7.6)	26.6 (t)	2.03 (dt, 7.3, 7.6)
6	124.4 (d)	5.12 (qt, 1.5, 7.6)	124.5 (d)	5.14 (qt, 1.5, 7.6)	124.2 (d)	5.14 (qt, 1.5, 7.6)
7	134.8 (s)		134.9 (s)		135.0 (s)	
8	39.5 (t)	1.96 (t, 7.4)	39.6 (t)	1.97 (t, 7.3)	39.7 (t)	1.90 (t, 7.3)
9	26.4 (t)	2.05 (dt, 7.6, 7.4)	26.5 (t)	2.08 (dt, 7.6, 7.3)	22.5 (t)	2.08 (dt, 7.6, 7.3)
10	125.1 (d)	5.11 (qt, 1.5, 7.6)	125.6 (d)	5.13 (qt, 1.5, 7.6)	124.4 (d)	5.13 (qt, 1.5, 7.6)
11	133.7 (s)		133.3 (s)		131.3 (s)	
12	38.0 (t)	2.07 (t, 7.3)	37.9 (t)	2.15 (t, 7.3)	39.7 (t)	2.15 (t, 7.3)
13	27.5 (t)	2.26 (dt, 7.3, 7.7)	27.4 (t)	2.44 (dt, 7.3, 7.7)	26.8 (t)	2.44 (dt, 7.3, 7.7)
14	145.0 (d)	6.83 (qt, 1.5, 7.7)	145.9 (d)	6.45 (qt, 1.5, 7.7)	124.3 (d)	5.12 (qt, 1.5, 7.7)
15	126.9 (s)		127.3 (s)		131.3 (s)	
16	173.3 (s)		195.3 (d)	9.37 (s)	25.7 (q)	1.64 (d, 1.5)
17	12.0 (q)	1.81 (d, 1.5)	19.2 (q)	1.74 (d, 1.5)	17.0 (q)	1.53 (d, 1.5)
18	15.9 (q)	1.58 (d, 1.5)	15.9 (q)	1.59 (d, 1.5)	15.9 (q)	1.59 (d, 1.5)
19	15.8 (q)	1.57 (d, 1.5)	15.8 (q)	1.57 (d, 1.5)	15.8 (q)	1.57 (d, 1.5)
20	24.0 (q)	1.24 (s)	24.1 (q)	1.26 (s)	24.0 (q)	1.19 (s)
1'	145.9 (s)		145.9 (s)		146.0 (s)	
2'	121.2 (s)		121.2 (s)		121.2 (s)	
3'	112.6 (d)	6.36 (d, 2.9)	112.6 (d)	6.38 (d, 2.9)	112.6 (d)	6.31 (d, 2.9)
4'	147.7 (s)		147.8 (s)		147.7 (s)	
5'	115.7 (d)	6.46 (d, 2.9)	115.6 (d)	6.48 (d, 2.9)	115.6 (d)	6.41 (d, 2.9)
6'	127.3 (s)		127.3 (s)		128.3 (s)	
7'	16.0 (q)	2.10 (s)	16.0 (q)	2.16 (s)	16.0 (q)	2.06 (s)

[a] Assignments are based on COSY, NOESY, CHSHF, and HMBC.
[b] Multiplicity and *J* in Hz are given in parentheses.

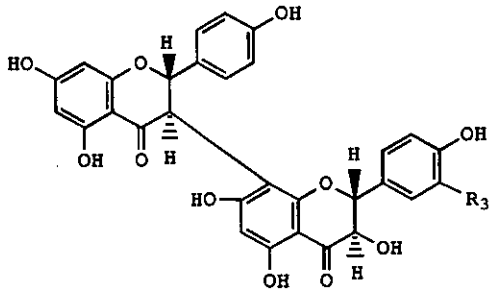


Garcinoic acid (**1**) R_1= H, R_2= COOH
Garcinal (**2**) R_1= H, R_2= CHO
δ-Tocotrienol (**3**) R_1= H, R_2= CH_3

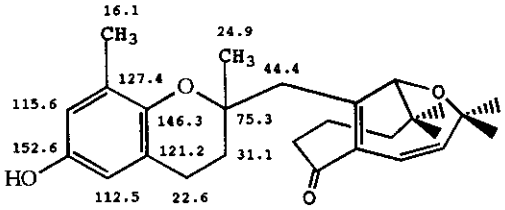
8: R_1= H, R_2= $COOCH_3$
9: R_1= MOM, R_2= $COOCH_3$
10: R_1= MOM, R_2= CH_2OH
11: R_1= MOM, R_2= CHO
12: R_1= MOM, R_2= CH_3



Garcinianin (**4**)



GB-1 (**5**): R_3= H
GB-2 (**6**): R_3= OH



Cystseirol A (**7**)

tocotrienol.[7,10] The compound (**3**) was identical with a product derived from garcinoic acid (**1**).

Antioxidant activities The antioxidant activities of the natural products (**1**, **2**, **3**) were shown to be 1.53, 1.52 and 1.47 times stronger, respectively, than that of *dl*-α-tocopherol by the Umezawa method.[11,12]

EXPERIMENTAL

Optical rotations were recorded on a JASCO DIP-181 digital polarimeter using a 100-mm length quartz cell at 25 °C. UV spectra were taken in CH_3OH with a JASCO UVIDEC-610 spectrophotometer. IR spectra were taken on a JASCO FT/IR-5000 spectrophotometer. MS spectra were obtained under EI conditions with a Hitachi M-80 spectrometer. ^1H-, ^{13}C-, two-dimensional (2D) NMR and difference NOE spectra were measured with JEOL α-400 and α-600 spectrometers. Absorbance at 532 nm was measured with a Spectronic 20 A Shimadzu B & L spectrophotometer.

Isolation Seeds of *Garcinia kola* (2.7 kg) collected in Nigeria were extracted with MeOH (10 L x 3) at rt to yield an extract (98.5 g). A part of the extract (10.2 g) was subjected to MPCC over silica gel (Katayama Chemicals Co. Ltd., Silica Gel 60, K 230, 150 g) using a mixture of chloroform and methanol (95 : 5) to give 6 fractions, 2.98 g, 0.13 g, 2.90 g, 1.08 g, 0.57 g, and 0.48 g.

***Isolation of chromanols* (1 - 3)** The least polar fraction (2.98 g) was subjected to column chromatography (CC) over silica gel (Fuji Silysia Co. Ltd., BW-820 MH, 50 g) using a mixture of hexane and acetone (90 : 10) to give 6 fractions, 681 mg, 299 mg, 273 mg, 548 mg, 1270 mg, and 292 mg. The second fraction (299 mg) was subjected to CC over silica gel (Fuji Silysia Co. Ltd., BW-820 MH, 8 g) using a mixture of hexane and acetone (90 : 10) followed by preparative TLC (Merck 5744, benzene and ethyl acetate (95 : 5)) to give δ-tocotrienol (**3**) (3.2 mg). The third fraction (273 mg) was subjected to CC over silica gel (Fuji Silysia Co. Ltd., BW-820 MH, 8 g) using a mixture of hexane and acetone (95 : 5) followed by preparative TLC (Merck 5744, hexane and acetone (90 : 10)) to give garcinal (**2**) (3.6 mg). The fifth fraction (1270 mg) was almost pure garcinoic acid (**1**).

Garcinoic acid (**1**) Pale yellowish oil: $[\alpha]_D$ 0° (*c* 0.15, $CHCl_3$; cell length 100 mm); UV (MeOH), λ 296 nm (ε 3800); IR (film), ν 3400, 1690, 1646 cm^{-1}; MS, *m/z* 426 (M^+, 34), 177 (46), 137 (100), 93 (54); ^1H NMR see Table 1; ^{13}C NMR see Table 1.

Garcinal (**2**) Pale yellowish oil: $[\alpha]_D$ 0° (*c* 0.03, $CHCl_3$; cell length 100 mm); UV (MeOH), λ 295 nm (ε 3300); IR (film), ν 3400, 1690, 1646 cm^{-1}: MS, *m/z* 410 (M^+, 6), 177 (19), 137 (45), 107 (22); ^1H NMR see Table 1; ^{13}C NMR see Table 1.

δ-Tocotrienol (**3**) Pale yellowish oil: $[\alpha]_D$ 0° (*c* 0.03, $CHCl_3$; cell length 100 mm); UV (MeOH), λ 297 nm (ε 3300); IR (film), ν 3400 cm^{-1}: MS, *m/z* 396 (M^+, 13), 177 (13), 137 (36); ^1H NMR see Table 1; ^{13}C NMR see Table 1.

Isolation of biflavonoids The third fraction (2.90 g) was subjected to column chromatography

(CC) over silica gel (Fuji Silysia Co. Ltd., BW-820 MH, 100 g) using a mixture of chloroform and methanol (90 : 10) to give garcinianin (**4**) (431 mg) and GB-1 (**5**) (1759 mg).[4,5] The fourth fraction (1.08 g) was subjected to CC over silica gel (Fuji Silysia Co. Ltd., BW-820 MH, 40 g) using a mixture of chloroform and methanol (90 : 10) to give GB-1 (**5**) (385 mg, totally 2144 mg) and GB-2 (**6**) (616 mg).[5]

Esterification A solution of garcinoic acid (**1**) (442 mg) in ether (10 mL) was treated with a solution of diazomethane at -78 °C for 5 min. After evaporation of the solvent, the residue was subjected to CC on SiO_2 (hexane and acetone (4 : 1)) to give a methyl ester (**8**) (367 mg, 80 %) as a colorless oil: IR (film), ν 3410, 1696, 1646 cm^{-1}; MS, *m/z* 440 [M^+]; ^1H NMR, δ 1.24 (3H, s, H-20), 1.57 (2H, m, H-4), 1.57 (3H, s, H-19), 1.61 (3H, s, H-18), 1.74 (2H, m, H-2), 1.81 (3H, d, *J*=1.5 Hz, H-17), 1.95 (2H, t, *J*=7.4, H-8), 2.02 - 2.07 (6H, m, H-5, 9, 12), 2.10 (3H, s, H-7'), 2.21 (2H, dt, *J*=7.3, 7.7, H-13), 2.67 (2H, t, *J*=6.6, H-1), 3.71 (3H, s, H-16), 4.53 (1H, s, -OH), 5.09 (2H, br t, *J*=7.6, H-6, 10), 6.36 (1H, d, *J*=2.9, H-3'), 6.46 (1H, d, *J*=2.9, H-5'), 6.72 (1H, dt, *J*=1.5, 7.3, H-14).

Etherification A mixture of the methyl ester (**8**) (448 mg, 1.018 mmol), *N,N*-diisopropylethylamine (526 mg, 4.078 mmol) and methoxymethyl chloride (328 mg, 4.075 mmol) in dichloromethane (60 mL) was stirred at rt overnight. The reaction mixture was successively washed with water and brine, and dried over Na_2SO_4. Purification by CC on SiO_2 (chloroform) afforded a MOM ether (**9**) (382 mg, 77 %) as a colorless oil: IR (film), ν 1711 cm^{-1}; MS, *m/z* 484 [M^+]; ^1H NMR, δ 1.24 (3H, s, H-20), 1.58 (2H, m , H-4), 1.58 (6H, s, H-18, 19), 1.74 (2H, m , H-2), 1.81 (3H, d, *J*=1.5, H-17), 1.95 (2H, t, *J*=7.4, H-8), 2.04 - 2.11 (6H, m, H-5, 9, 12), 2.13 (3H, s, H-7'), 2.24 (2H, dt, *J*=7.3, 7.7, H-13), 2.70 (2H, t, *J*=6.6, H-1), 3.46 (3H, s, H-4') , 3.71 (3H, s, H-16) , 5.05 (2H, s, H-4'), 5.11 (2H, qt , *J*=1.5, 7.6, H-6, 10), 6.58 (1H, d, *J*=2.9, H-3'), 6.66 (1H, d, *J*=2.9, H-5'), 6.72 (1H, qt , *J*=1.5, 7.3, H-14).

Reduction A solution of the MOM ether (**9**) (349 mg, 0.721 mmol) in THF (40 mL) was treated with $LiAlH_4$ (110 mg, 2.895 mmol) at rt overnight. After addition of methanol (35 mL), the mixture was filtered through celite. The filtrate was diluted with ethyl acetate (100 mL) and then successively washed with water and brine, and dried over Na_2SO_4. Purification by CC on SiO_2 (hexane and ethyl acetate (5 : 1)) afforded a primary alcohol (**10**) (222 mg, 70 %) as a colorless oil: IR (film), ν 3372 cm^{-1}; MS, *m/z* 456 [M^+]; ^1H NMR, δ 1.24 (3H, s, H-20), 1.55 (2H, m, H-4), 1.55 (3H, s, H-19), 1.57 (3H, s, H-18), 1.64 (3H, s, H-17), 1.75 (2H, m, H-2), 1.93 - 2.11 (10H, m, H-5, 8, 9, 12, 13), 2.12 (3H, s, H-7'), 2.70 (2H, t, *J*=7.0, H-1), 3.46 (3H, s H-4'), 3.97 (2H, s, H-16), 5.05 (2H, s, H-4'), 5.09 (1H, qt , *J*=1.5, 7.3, H-10), 5.11 (1H, qt , *J*=1.5, 7.3, H-6), 5.36 (1H, dt, *J*=1.5, 7.3, H-14), 6.58 (1H, d, *J*=2.9, H-3'), 6.62 (1H, d, *J*=2.9, H-5').

Oxidation A solution of the alcohol (**10**) (199 mg, 0.436 mmol) in dichloromethane (25 mL) was treated with pyridinium dichromate (246 mg, 0.654 mmol) at rt for 4 h. The mixtute was filtered through celite. After evaporation of the solvent, the residue was subjected to CC on SiO_2 (hexane and ethyl acetate (10 : 1)) to give a MOM-aldehyde (**11**) (160 mg, 80 %) as a colorless oil: IR (film), ν 1684, 1642 cm^{-1};

MS, *m/z* 454 [M$^+$]; ^{1}H NMR, δ 1.24 (3H, s, H-20), 1.57 (3H, s, H-19), 1.58 (2H, m, H-4), 1.59 (3H, s, H-18), 1.72 (3H, d, *J*=1.5, H-17), 1.72 (2H, m, H-2), 1.95 (2H, t, *J*=7.3, H-8), 2.03 - 2.11 (6H, m, H-5, 9, 12), 2.12 (3H, s, H-7'), 2.42 (2H, dt , *J*=7.3, 7.7 , H-13), 2.70 (2H, t , *J*=7.0, H-1), 3.46 (3H, s, H-4'), 5.05 (2H, s, H-4'), 5.11 (1H, qt , *J*=1.5, 7.3, H-10), 5.12 (1H, qt , *J*=1.5, 7.3, H-6), 6.44 (1H, dt, *J*=1.5, 7.3 , H-14), 6.57 (1H, d, *J*=2.9, H-3'), 6.65 (1H, d, *J*=2.9, H-5'), 9.35 (1H, s, H-16).

Deprotection The MOM-aldehyde (**11**) (13 mg, 0.029 mmol) in a mixture of chloroform and methanol (1 : 1) (1.5 mL) was treated with conc. hydrochloric acid (2 drops) under reflux for 1 h. The mixture was diluted with chloroform (10 mL) and then successively washed with water and brine, and dried over Na_2SO_4. Purification by preparative TLC on SiO_2 (Merck 5744, hexane and ethyl acetate (3 : 1)) afforded an aldehyde (8 mg, 67 %) as a colorless oil. The aldehyde was identical with garcinal (**2**).

Wolff-Kishner reduction A mixture of the aldehyde (**11**) (245 mg, 0.540 mmol), 80 % hydrazine hydrate (1.2 mL, 19 mmol) and potassium hydroxide (585 mg, 10.4 mmol) in triethylene glycol (20 mL) was stirred at 120 °C for 2 h and then at 190 °C for 2 h. The mixture was diluted with ethyl acetate (50 mL) and then successively washed with water and brine, and dried over Na_2SO_4. Purification by CC on SiO_2 (hexane and ethyl acetate (10 : 1)) afforded a MOM-ether (**12**) (73 mg, 33 %) as a colorless oil: IR (film), ν 1642 cm^{-1}; MS, *m/z* 440 [M$^+$]; ^{1}H NMR, δ 1.24 (3H, s, H-20), 1.53 (2H, m, H-4), 1.55 (6H, s, H-18, 19) , 1.58 (3H, s, H-16), 1.67 (2H, m, H-2), 1.69 (3H, s , H-17), 1.88 - 2.10 (10H, m, H-5, 8, 9, 12, 13), 2.12 (3H, s, H-7'), 2.70 (2H, t , *J*=6.8, H-1), 3.46 (3H, s, H-4'), 5.05 (2H, s, H-4'), 5.07 (1H, m , H-10), 5.09 (1H, m , H-6), 5.11 (1H, m , H-14), 6.57 (1H, d, *J*=2.9, H-3'), 6.66 (1H, d, *J*=2.9, H-5').

Deprotection The MOM-ether (**12**) (73 mg, 0.166 mmol) in a mixture of chloroform and methanol (1 : 1) (8 mL) was treated with conc'd hydrochloric acid (2 drops) under reflux for 1 h. The mixture was diluted with chloroform (10 mL) and then successively washed with water and brine, and dried over Na_2SO_4. Purification by preparative CC on SiO_2 (hexane and ethyl acetate (10 : 1)) afforded a phenol (36 mg, 70 %) as a colorless oil. The phenol was identical with δ-tocotrienol (**3**).

Antioxidant activity A mixture of 0.2M Tris-HCl buffer (pH 7.4), 8mM sodium arachidonoate, a methanolic solution of an antioxidant, 1mM bleomycin and 1.08mM $FeSO_4$ (each 0.1 mL) was incubated at 37 °C for 5 min, followed by addition of 0.2N HCl (10 μL). After addition of 0.5% thiobarbituric acid (0.2 mL), the solution was incubated at 37 °C for 30 min. Then, H_2O (0.4 mL) and BuOH (1 mL) were added, and the mixture was vigorously shaken and centrifuged at 3000 rpm for 10 min. The absorbance of the BuOH layer at 532 nm was measured by spectrophotometry.

ACKNOWLEDGEMENT

We are grateful to Professor T. Kato (Faculty of Science, Science University of Tokyo) for valuable informations of δ-tocotrienol, Dr. K. Kato (Nihon Kayaku Co. Ltd., Tokyo) for providing bleomycin, and Japan Private School Promotion Foundation for financial support.

REFERENCES AND NOTES

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Received, 13th May, 1997