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CONSTITUENTS OF GREEN AND RIPENED FRUIT OF *GARCINIA SUBELLIPTICA*

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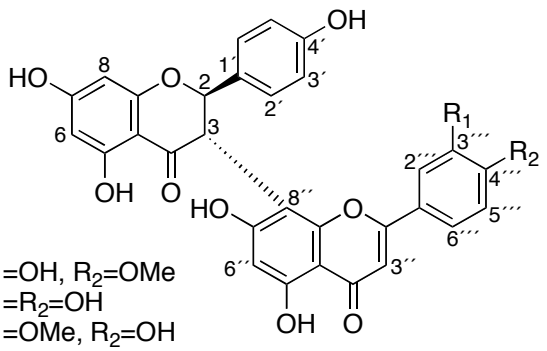
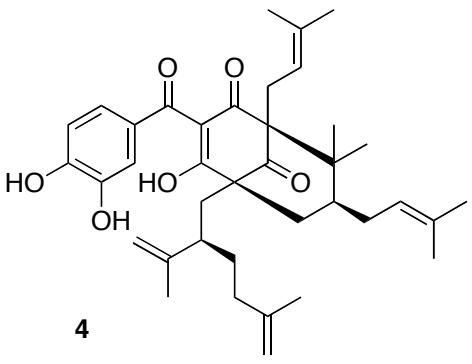
Abstract – The structure of one new C-3/C-8˝-biflavonoid from *Garcinia subelliptica* fruit, (+)-4˝˝˝-*O*-methylfukugetin, and the comparison of the constituents from green and ripened *G. subelliptica* fruit are described.

INTRODUCTION

The plants of the *Garcinia* genus (Guttiferae) are a rich source of biologically and pharmacologically active substances, and some of them are used as traditional medicines in areas of Asia and Africa.[1] Of about 400 *Garcinia* spp., only *G. subelliptica* originates in Japan, mainly in Okinawa and Ogasawara Islands.[2] In Japanese, *G. subelliptica* is known as 'Fukugi,' which means 'a tree bringing happiness.' The trees are common in Okinawa as roadside and garden trees, and a yellow pigment from the bark is a dye for a traditional Okinawan fabric. Although many *Garcinia* spp. fruits are edible, such as the mangosteen from *G. mangostana*, the fruits of *G. subelliptica* are inedible. Furthermore, fallen fruits give off a strong and unpleasant smell, which is disliked by the islanders.



1: R_1=OH, R_2=OMe
2: R_1=R_2=OH
3: R_1=OMe, R_2=OH
5: R_1=H, R_2=OH



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In the course of searching for new medicinal plant resources, we have focused on the fruits of *G. subelliptica* in Okinawa. There has been little chemical study of the fruit, although the chemical constituents of stem, wood, bark, and root have been much studied, and various flavonoids, xanthones, and benzophenones were reported.[3–5] Two triterpenes were isolated from *G. subelliptica* seed grown in Taiwan.[6] We report here on two topics, the structure of a new C-3/C-8˝-biflavonoid, and the comparison of the constituents from green and ripened *G. subelliptica* fruit.

RESULTS AND DISCUSSION

Green and ripened *G. subelliptica* fruits were collected in Okinawa, and separated into flesh and seed. The flesh and the seed of the green and ripened fruit were respectively extracted with methanol to give totally four extracts. Each extract was separated and purified by a combination of silica gel column chromatography, preparative thin layer chromatography and reversed phase HPLC, to give one new compound (**1**), (+)-4˝˝˝-*O*-methylfukugetin, and three known compounds (**2**, **4**, and **5**).

[1] Structure of (+)-4˝˝˝-*O*-methylfukugetin (1)

(+)-4˝˝˝-*O*-Methylfukugetin (**1**), $[\alpha]_D$ +171.2° (*c* 0.09, MeOH) had the molecular formula $C_{31}H_{22}O_{11}$ determined by high-resolution FABMS. The IR spectrum showed the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1652 and 1636 cm^{-1}) groups. The ^1H NMR spectrum in DMSO-d_6 of **1** exhibited signals for one set of *ortho*-coupled aromatic hydrogens at δ 6.39 and 7.14 (each 2H, d, *J* = 8.0 Hz), one set of *meta*-coupled aromatic hydrogens at δ 5.97 and 5.98 (each 1H, d, *J* = 2.0 Hz), one set of ABX-type (1,2,4-trisubstituted) aromatic hydrogens at δ 7.10 (1H, d, *J* = 8.0 Hz); 7.45 (1H, d, *J* = 2.0 Hz); 7.55 (1H, dd, *J* = 8.0, 2.0 Hz), and two uncoupled aromatic hydrogens at δ 6.24 and 6.65 (each 1H, s) as shown in Table 1. Furthermore, there were two aliphatic hydrogens that coupled each other at δ 4.90, 5.73 (each 1H, d, *J* = 11.9 Hz), a signal of methoxyl group at δ 3.87 (3H, s), and two interchangeable protons by addition of deuterium oxide at δ 12.2 and 13.0 (each 1H, s) were observed. As these spectral data were very similar to those of the 3,8˝-biflavonoid, (+)-morelloflavone (**2**) isolated from the same plant,[7] compound **1** seemed to be a methylated analogue of **2** and a regioisomer of (+)-3˝˝˝-*O*-methylfukugetin (**3**) based on a methoxyl group.[4a] The linkage of two flavonoid moieties at C-3 – C-8˝ was confirmed by the following correlation on HMBC spectrum, H-2´(6´) / C-2, H-2˝˝(6˝˝) / C-2˝, HO-5 / C-5, HO-5˝ / C-5˝, and H-3, H-6˝ / C-8˝. On the other hand, the correlation between MeO-H and C-4˝˝˝ indicated the location of methoxyl group to be at C-4˝˝˝. Furthermore, NOE between MeO-H and H-5˝˝˝ also supported the result.

The stereochemistry between C-2 and C-3 of **1** was determined on the basis of ^1H NMR spectral data, as follows. The coupling constant (*J* = 11.9 Hz) of H-2 and H-3 suggested the stereochemistry between H-2

Table 1. NMR Data of **1** and **2** in DMSO-d_6

	1			**2**	
Positions	^{13}C NMR	^{1}H NMR	HMBC	^{13}C NMR	^{1}H NMR
2	80.9	5.73 (d, *J* = 11.9)	H-2'(6')	81.0	5.71 (d, *J* = 12.0)
3	48.3	4.90 (d, *J* = 11.9)		48.4	4.89 (d, *J* = 12.0)
4	196.1		H-3	196.3	
5	163.7		5-OH	161.8	
6	96.1	5.97 (d, *J* = 2.0)	5-OH	95.4	5.97 (br. s)
7	162.7		H-6	163.6	
8	95.2	5.98 (d, *J* = 2.0)		96.3	5.97 (br. s)
4a	101.4		5-OH	101.6	
8a	166.5		H-8	166.6	
1'	128.1		H-3, H-3'(5')	128.2	
2', 6'	128.6	7.14 (d, *J* = 8.0)		128.6	7.15 (d, *J* = 8.3)
3', 5'	114.1	6.39 (d, *J* = 8.0)		114.5	6.39 (d, *J* = 8.3)
4'	157.3		H-2'(6'), H-3'(5')	157.4	
2''	162.8		H-3'', H-2''', H-6'''	163.8	
3''	102.8	6.65 (s)		102.3	6.58 (s)
4''	181.6		H-3''	181.7	
5''	160.5		H-6'', 5''-OH	160.6	
6''	98.6	6.24 (s)	5''-OH	98.7	6.23 (s)
7''	161.9		H-3, H-6''	162.9	
8''	100.5		H-3, H-6''	100.6	
4a''	103.1		H-3'', H-6'', 5''-OH	103.2	
8a''	155.2		H-3	155.3	
1'''	125.5		H-2''', H-5'''	121.1	
2'''	112.8	7.45 (d, *J* = 2.0)		113.4	7.42 (br s)
3'''	146.7		H-2''', H-5'''	145.7	
4'''	151.1		H-2''', H-6''', -OMe	149.8	
5'''	112.2	7.10 (d, *J* = 8.0)		116.2	6.91 (d, *J* = 8.1)
6'''	118.9	7.55 (dd, *J* = 8.0, 2.0)		119.4	6.97 (br d, *J* = 8.0)
OMe	55.6	3.87 (s)			
5-OH		12.2 (s)			12.25 (s)
5''-OH		13.0 (s)			13.07 (s)

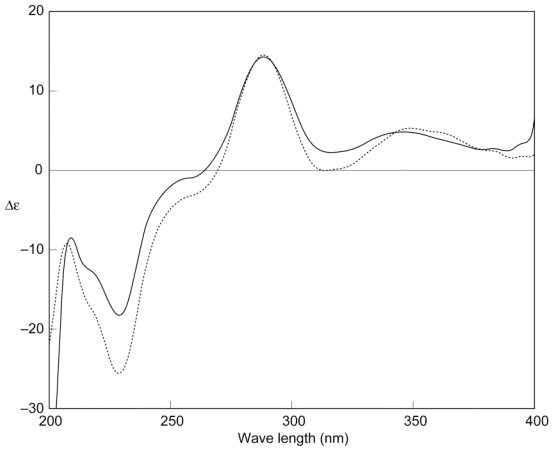

Figure 1. CD spectra of (+)-4´´´-*O*-methylfukugetin (**1**) and (+)-morelloflavone (**2**) in methanol ——: **1**, ·····: **2**

and H-3 to be *trans*. Moreover, the absolute stereochemistry of (+)-morelloflavone (**2**) has been already determined by CD spectrum to be 2*R*,3*S*.[7a] The CD curve of **1** showed a very similar pattern to **2** as shown in Fig. 1. Thus, the absolute configuration of **1** was confirmed to be 2*R*,3*S*.

[2] Comparison of the constituents from the green and ripened fruits (Table 2)

2-1: The constituents of the flesh:

i) From green fleshes: (+)-xanthochymol (**4**), a prenylated benzophenone derivative, was given in 0.025% yield based on the basis of the raw green flesh.

ii) From ripened fleshes: (+)-xanthochymol (**4**), was given in 0.146% yield based on the basis of the raw ripened flesh.

The yield of (+)-xanthochymol (**4**) from ripened fleshes is about 6 times more than that from green ones.

2-2: The constituents of the seed:

i) From green seed: (+)-morelloflavone (**2**), a biflavonoid, was isolated in 0.026% yield based on the basis of the raw green seed.

ii) From ripened seed: three 3,8´´-biflavonoids, (+)-morelloflavone (**2**), (+)-4´´´-*O*-methylfukugetin (**1**) and (+)-volkensiflavone (**5**) were isolated in 0.265%, 0.013% and 0.031%, respectively, based on the basis of the raw ripened seed.

This is the first report of the isolation of (+)-volkensiflavone (**5**) from *G. subelliptica*. The yield of (+)-morelloflavone (**2**) from ripened seeds is about 10 times more than that from the green ones.

The biologically interesting activities such as antioxidant, and HIV-inhivbitory activities, on (+)-morelloflavone (**2**) and (+)-xanthochymol (**4**) have been reported.[8,9] This research showed that the ripened fruits of *G. subelliptica* have a possibility to be one of new natural medicinal resources, although they are disliked with a strongly bad smell.

Table 2. Yields of the isolated constituents from the flesh and seed[a]

		1	**2**	**4**	**5**
Flesh	from green fruits	—	—	0.025	—
	from ripened fruits	—	—	0.146	—
Seed	from green fruits	—	0.026	—	—
	from ripened fruits	0.013	0.265	—	0.031

[a] Yields by weight were calculated on the basis of each raw material.

EXPERIMENTAL

General

IR spectra were recorded on JASCO FT/IR-410 spectrometers. Optical rotations and CD spectra were measured with a JASCO P-1020 polarimeter (cell length 100 mm) and a JASCO J-725 circular dichroism spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on JEOL α–600 (^{1}H: 600 MHz and ^{13}C: 150 MHz) and JEOL α-400 (^{1}H: 400 MHz and ^{13}C: 100 MHz) spectrometers. Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to the solvent signal (DMSO-d_6: δ_H 2.49 and δ_C 39.5) as an internal standard. LR and HR FAB-MS were obtained with JEOL JMS HX-110 using *m*-nitrobenzyl alcohol as matrix. Analytical TLC and preparative TLC were performed on silica gel 5715 and 5744 (Merck), respectively. Column chromatography was carried out on silica gel BW-820MH (Fuji Silysia Chemicals, Co. Ltd.).

Extraction

The green fruits (102 g) collected at Okinawa Island in August, 2001 were separated to the flesh (75 g) and the seed (21 g). The chopped flesh and the minced seed were respectively extracted with methanol (each 500 mL, 3 times) to give the corresponding extracts, Gf (4.5 g) and Gs (1.5 g).

The ripened fruits (157 g) collected at Okinawa Island in November, 2001 were separated to the flesh (102 g) and the seed (51 g). The chopped flesh and the minced seed were respectively extracted with methanol (each 800 mL, 3 times) to give the corresponding extracts, Rf (8.0 g) and Rs (3.9 g).

Isolation

Gf (4.5 g) was chromatographed over SiO_2 (50 g) using a mixture of $CHCl_3$ and MeOH (95 : 5 (150 mL), 90 : 10 (150 mL), 80 : 20 (150 mL)) to give 6 fractions (Fr-1: 23 mg, Fr-2: 446 mg, Fr-3: 339 mg, Fr-4: 106 mg, Fr-5: 176 mg, Fr-6: 377 mg). Fr-2 (446 mg) was chromatographed over SiO_2 (10 g) again using a mixture of benzene and AcOEt (95:5) to give 5 fractions (Fr-2-1: 2 mg, Fr-2-2: 10 mg, Fr-2-3: 13 mg, Fr-2-4: 160 mg, Fr-2-5: 6 mg). Fr-2-4 (160 mg) was further separated with reversed phase medium pressure liquid chromatography (MPLC) using ODS column (Develosil Lop ODS, ϕ30 x 300 mm, Nomura Chemical, Aichi) and a mixed solvent of MeOH and H_2O (90 : 10) to give 3 fractions (Fr-2-4-1: 4 mg, Fr-2-4-2: 78 mg, Fr-2-4-3: 60 mg). Fr-2-4-2 (60 mg) was finally purified with preparative TLC using a mixture of hexane and EtOAc (75 : 25) to give (+)-xanthchymol (**4**) (19 mg), which was identified by comparison with the reported spectral data.[10]

Gs (1.5 g) was chromatographed over SiO_2 (30 g) using a mixture of $CHCl_3$ and MeOH (90 : 10) to give 5 fractions (Fr-1: 53 mg, Fr-2: 107 mg, Fr-3: 123 mg, Fr-4: 312 mg, Fr-5: 672 mg). Fr-3 (123 mg) was separated and then purified with preparative TLC using a mixture of $CHCl_3$ and MeOH (85 : 15) to give (+)-morelloflavone (**2**) (55 mg), which was identified by comparison with the spectral data.[7]

Rf (8.0 g) was chromatographed over SiO_2 (200 g) using a mixture of $CHCl_3$ and MeOH (95 : 5) to give 4 fractions (Fr-1: 45 mg, Fr-2: 274 mg, Fr-3: 144 mg, Fr-4: 5.9 g). Fr-2 (274 mg) was subjected to column chromatography and then to preparative TLC using a mixture of $CHCl_3$ and MeOH (95 : 5) to give (+)-xanthchymol (**4**) (149 mg), which was identified by comparison with the spectral data.[10]

Rs (3.9 g) was chromatographed over SiO_2 (100 g) using a mixture of $CHCl_3$ and MeOH (90 : 10) to give 7 fractions (Fr-1: 193 mg, Fr-2: 313 mg, Fr-3: 108 mg, Fr-4: 96 mg, Fr-5: 28 mg, Fr-6: 2052 mg, Fr-7: 67 mg). Fr-6 (2052 mg) was chromatographed over SiO_2 (50 g) using a mixture of $CHCl_3$ and hexane (90 : 10) to give 3 fractions (Fr-6-1: 29 mg, Fr-6-2: 39 mg, Fr-6-3: 1949 mg). Fr-6-3 (1949 mg) was rechromatographed over SiO_2 (60 g) using a mixture of $CHCl_3$ and MeOH (95 : 5) to give 5 fractions (Fr-6-3-1: 19 mg, Fr-6-3-2: 19 mg, Fr-6-3-3: 230 mg, Fr-6-3-4: 480 mg, Fr-6-3-5: 1131 mg). Fr-6-3-3 (230 mg) was separated and then purified with preparative TLC using a mixture of $CHCl_3$ and MeOH (90 : 10) to give (+)- 4´´´-*O*-methylfukugetin (**1**) (6.5 mg), (+)-volkensiflavone (**5**) (16 mg) and (+)-morelloflavone (**2**) (135 mg), which were identified by comparison with the spectral data.[11]

(+)-4´´´-*O*-methylfukugetin (1), $[\alpha]_D$ +171.2° (*c* 0.09, MeOH); UV λ_{max} (MeOH) (nm (log ε)) 341.0 (4.8), 288.0 (5.0), 228.5 (sh., 5.2); CD λ (MeOH) nm (Δε) 345.4 (4.84), 288.4 (14.34), 228.0 (–18.14); IR ν_{max} ($CHCl_3$) 3400, 1652, 1636 cm^{-1}; ^1H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) data are shown in Table 1; HREI-MS: *m*/*z* 571.1232 $[M+H]^+$ (571.1240 calculated for $C_{31}H_{23}O_{11}$).

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REFERENCES

1. a) '*Genshoku Chugoku Honzou Zukan*,' Vol. 5, ed. by X. Qian, Yuko-Sha, Kyoto, 1986, p. 80, and Vol. 7, p 282; b) M. Niwa, K. Terashima, J. Ito, and M. Aqil, *Heterocycles*, 1994, **38**, 1071.
2. S. Hatsushima and C. Nakajima, *Flowers of the Ryukyu Islands*, Kodansya, Tokyo, 1979, p 259.
3. a) Y. Fukuyama, A. Kamiyama, Y. Mima, and M. Kodama, *Phytochemistry,* 1991, **30**, 3433; b) H. Minami, M. Kinoshita, Y. Fukuyama, M. Kodama, T. Yoshizawa, M. Sugiura, K. Nakagawa, and H. Tago, *Phytochemistry,* 1994, **36**, 501; c) H. Minami, E. Takahashi, Y. Fukuyama, M. Kodama, T. Yoshizawa, and K. Nakagawa, *Chem. Pharm. Bull.,* 1995, **43**, 347; d) H. Minami, E. Takahashi, M. Kodama, and Y. Fukuyama, *Phytochemistry,* 1996, **41**, 629; e) H. Minami, A. Kuwayama, T. Yoshizawa, and Y. Fukuyama, *Chem. Pharm. Bull.,* 1996, **44**, 2103; f) H. Minami, K. Hamaguchi, M. Kubo, and Y. Fukuyama, *Phytochemistry,* 1998, **49**, 1783; g) Y. Fukuyama, A. Kaneishi, N. Tani,

and M. Kodama, *Phytochemistry,* 1993, **33**, 483; h) Y. Fukuyama, A. Kuwayama, and H. Minami, *Chem. Pharm. Bull.,* 1997, **45**, 947; i) Y. Fukuyama, H. Minami, and A. Kuwayama, *Phytochemistry,* 1998, **49**, 853.

4. a) M. Konoshima, Y. Ikeshiro, A. Nishinaga, T. Matsuura, T. Kubota, and H. Sakamoto, *Tetrahedron Lett.*, 1969, **10**, 121; b) M. Konoshima, Y. Ikeshiro, S. Miyahara, and K. Yen *Tetrahedron Lett.*, 1970, **11**, 4203; c) M. Konoshima and Y. Ikeshiro, *Tetrahedron Lett.*, 1970, 1717.
5. a) M. Iinuma, H. Tosa, T. Tanaka, R. Shimano, F. Asai, and Y. Yoshimori, *Phytochemistry,* 1994, **35**, 1355; b) M. Iinuma, H. Tosa, T. Tanaka, F. Asai, and R. Shimano, *Heterocycles,* 1995, **40**, 279; c) M. Iinuma, H. Tosa, T. Tanaka, F. Asai, and R. Shimano, *Phytochemistry,* 1995, **38**, 247; d) M. Iinuma, H. Tosa, T. Tanaka, F. Asai, and R. Shimano, *Phytochemistry*, 1995, **39**, 945.
6. a) C. Lin, C. Kiang, C. Lu, R. Wu, and K. Lee, *Chem. Commun.*, 1996, 1315; b) M. Chung, H. Su, and C. Lin, *J. Nat. Prod.*, 1998, **61**, 1015.
7. a) X.-C. Li, A. S. Joshi, B. Tan, H. N. ElSohly, L. A. Walker, J. K. Zjawiony, and D. Ferreira, *Tetrahedron*, 2002, **58**, 8709; b) C. G. Karanjgoakar, P. V. Radhakrishnan, and K. Venkataraman, *Tetrahedron Lett.*, 1967, **33**, 3195.
8. M. Iinuma, H. Tosa, T. Tanaka, S. Kanamaru, F. Asai, Y. Kobayashi, K. Miyauchi, and R. Shimano, *Biol. Pharm. Bull.,* 1996, **19**, 311.
9. Y.-M. Lin, H. Anderson, M. T. Flavin, and Y. H. S. Pai, *J. Nat. Prod.*, 1997, **60**, 884.
10. C. G. Karanjgoakar, A. V. Rama Rao, K. Venkataraman, S. S. Yemul, and K. J. Palmer, *Tetrahedron Lett.*, 1973, **14**, 4977.
11. B. Jackson, H. D. Locksley, and F. Scheinmann, *Phytochemistry,* 1970, **9**, 221.