GARCINIANIN, A NOVEL BIFLAVONOID FROM THE ROOTS OF *GARCINIA KOLA*

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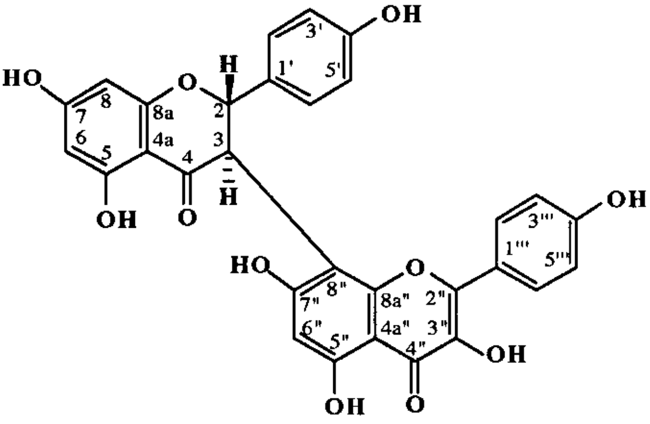
Abstract —— A novel biflavonoid named garcinianin was isolated from the roots of *Garcinia kola* (Guttiferae) and the structure was characterized as a C-3/C-8"-naringenin/kaempferol dimer by spectroscopic methods. Garcinianin is present as a mixture of two atropisomers (relative ratio; 1 : 1.2) at 25 °C. Garcinianin is the first example of a flavanone-flavonol biflavonoid.

Our previous studies on the constituents of the roots of *Garcinia kola* Heckel (Guttiferae) collected in Nigeria resulted in the isolation of novel arylbenzofurans and arylbenzopyran.[1,2] Our continuous study on the constituents of the above plant led to the isolation of a novel C-3/C-8"-flavanone/flavonol dimer named garcinianin (**1**) together with a known flavanone/flavanol, GB-1 (**2**) from the bark of the same plant.[3] In this paper we wish to describe the isolation and structure of garcinianin (**1**).

Isolation The water soluble fraction treated with chloroform described in the previous paper[1] was partitioned between ethyl acetate and water, and then a part of the organic fraction was subjected to by repeated medium pressure column chromatographies on silica gel using a gradient solvent system of chloroform and methanol to give garcinianin (**1**) and GB-1 (**2**) as optically inactive pale brownish powder, respectively. GB-1 was identified by comparison with the spectral data in the literature.[3,4]



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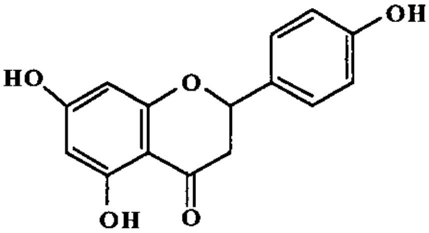

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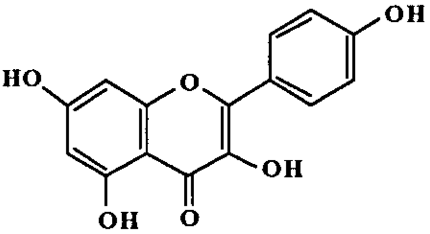

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Structure of Garcinianin (1) Garcinianin was found to have the molecular formula $C_{30}H_{20}O_{11}$ determined by the high resolution FABms. However, ^{13}C nmr of **1** at 25 °C showed fifty two signals forming twenty six pairs (Table 1). ^{1}H Nmr of **1** at 25 °C showed also the signals forming their respective pairs. These nmr data suggest garcinianin (**1**) to be a mixture of two isomers (relative ratio; 1 : 1.2).[5] Actually, however, these signals are due to two atropisomers of **1**, as follows. ^{1}H Nmr spectrum of **1** was run in the variable temperature. The sharp paired signals given at 25 °C became broad at 75 °C, and then became the sharp signals making no pair at 150 °C as shown in Figure 1. The spectrum at 150 °C showed the presence of such as four pairs of *ortho*-coupled signals, two pairs of *meta*-coupled signals and two pairs of hydrgogen-bonded signals, corresponding to eight protons, two protons and two protons, respectively. ^{13}C Nmr at 150 °C showed twenty six signals making no pair (Table 1) (eighteen signals and thirty four signals due to methine carbon and quarternary carbon, respectively). The strong resemblance of the data to those of naringenin (**3**)[6] and of kaempferol (**4**)[7] suggested that garcinianin is a C-3/C-8" or C-3/C-6"-linked naringenin/kaempferol dimer. The stereochemistry at C-2/C-3 positions is *trans* as judged from the *J*-value ($\delta_{H\text{-}3}$ 4.89, 1H, J=12.2 Hz, and $\delta_{H\text{-}2}$ 5.73, 1H, J=12.2 Hz). The linked position C-8" or C-6" was determined by the HMBC experiment at 25 °C, as follows. Correlations between C-6" (δ_C 97.5 and 98.1) and HO-5" (δ_H 12.49 and 12.57) forming hydrogen-bond with the carbonyl group, and between C-8" (δ_C 99.6 and 100.3) and H-3 (δ_H 4.98 and 4.92) were observed. This led garcinianin to be

Table 1. ^{13}C Nmr data of garcinianin (**1**), naringenin (**3**)[6] and kaempferol (**4**)[7] in DMSO-d_6

	Garcinianin (**1**)			Naringenin (**3**)*	Kaempferol (**4**)*
Position	25 °C		150 °C		
2	80.8	81.7	80.9	78.4	
3	47.3	48.0	47.6	42.1	
4	196.4	196.7	195.3	196.2	
5	163.8	163.9	163.2	163.6	
6	96.1	96.4	95.9	95.9	
7	166.5	167.1	166.1	166.7	
8	95.2	95.4	94.7	95.1	
4a	101.3	101.8	102.5	101.8	
8a	162.8	162.9	162.3	162.9	
1'	127.7	128.3	127.7	128.9	
2', 6'	128.4	128.8	127.4	128.2	
3', 5'	114.4	114.6	114.1	115.2	
4'	157.3	157.6	156.8	157.8	
2"	146.6	147.1	146.6		146.8
3"	135.2	135.6	134.6		135.6
4"	175.8	176.0	175.2		175.9
5"	159.6	159.7	159.1		160.7
6"	97.5	98.1	97.6		98.2
7"	162.2	162.4	161.2		163.9
8"	99.6	100.3	99.7		93.5
4a"	102.4	103.1	101.3		103.1
8a"	153.4	154.2	153.5		156.2
1'''	121.4	121.5	121.1		121.7
2''', 6'''	129.2	129.7	128.5		129.5
3''', 5'''	115.1	115.5	114.8		115.4
4'''	159.1	159.2	158.5		159.2

* The spectra were taken at ambient temperature.

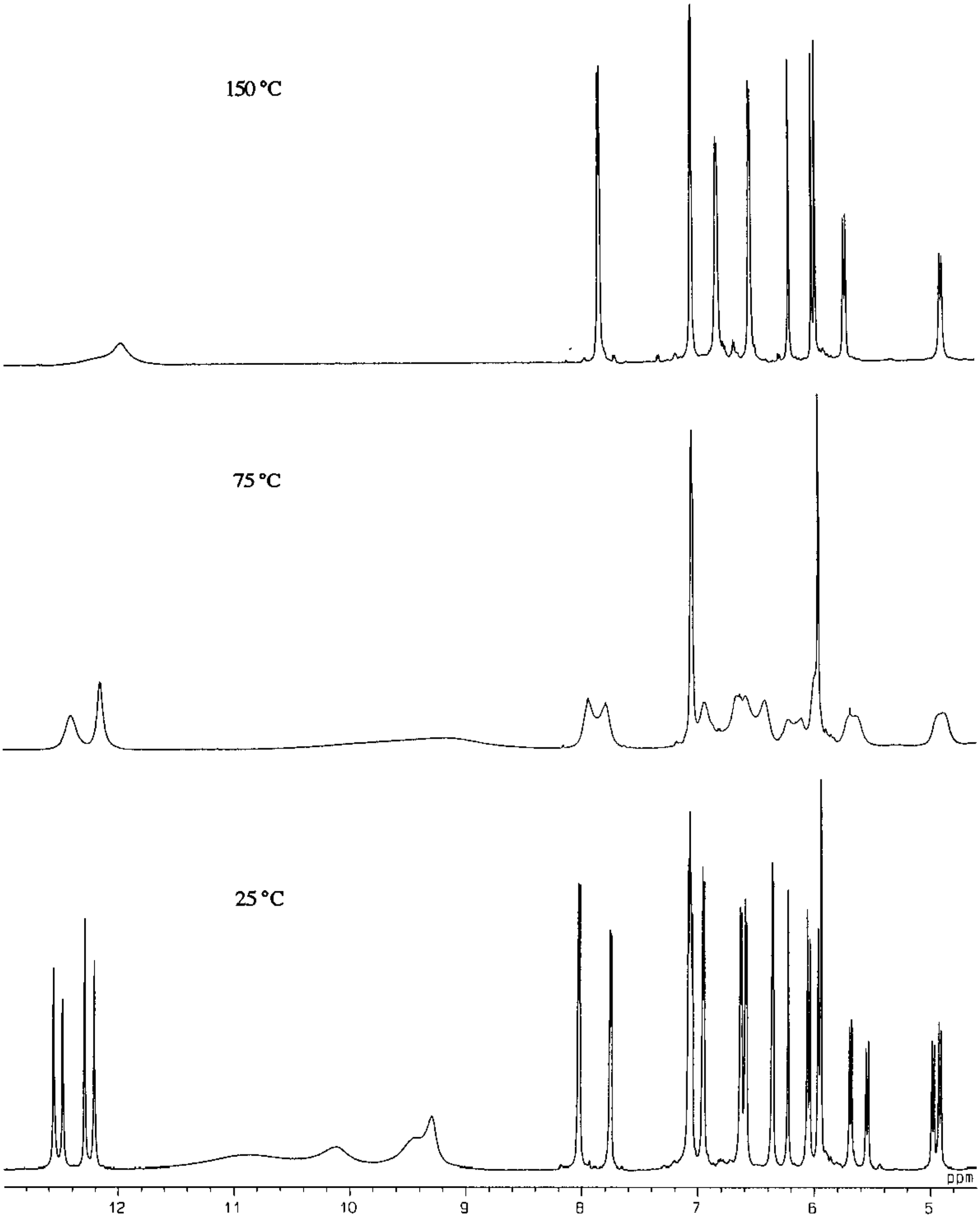

Figure 1. ^{1}H Nmr spectra of garcinianin (1) measured at 25, 75 and 150 °C

the dimer (**1**) of naringenin and kaempferol linked at C-3 and C-8 positions, respectively. Garcinianin is the first example of a flavanone/flavonol biflavonoid.

EXPERIMENTAL

Ir spectra were taken on a JASCO FT/IR-5000 infrared spectrophotometer. Uv spectrum was recorded on a JASCO UVIDEC-610 spectrophotometer. Optical rotation was recorded on a JASCO DIP-181 polarimeter at 25°C. ^{1}H and ^{13}C nmr spectra were recorded on a JEOL A-600 (600 and 125 MHz, respectively) spectrometer. Chemical shifts are presented in terms of δ_H and δ_C (ppm) with DMSO (2.49 and 39.5 ppm, respectively) in the solvent DMSO-d_6 as an internal standard. Coupling constants are given (*J* in Hz) in parentheses. Mass spectra were recorded on a JEOL HX-110 spectrometer.

Isolation

The water soluble fraction treated with chloroform described in the previous paper[1,2] was partitioned between ethyl acetate and water to give an EtOAc soluble fraction (9.1 g). A part of the EtOAc soluble fraction (5.1 g) was roughly fractinated by CC on silica gel (Katayama Chemical, Osaka, K70 150 g) with $CHCl_3$ - MeOH (9 : 1) to give 3 fractions (Fr.1 0.26 g; Fr. 2 3.9 g; Fr. 3 0.47 g). The second fraction (3.9 g) was separated by medium pressure CC on silica gel (Katayama Chemical, Osaka, K230 150 g) with $CHCl_3$ - MeOH (19 : 1) to give 5 fractions (Fr.1 0.09 g; Fr. 2 0.95 g; Fr.3 2.15 g; Fr. 4 0.71 g; Fr. 5 0.30 g). The third fraction (2.15 g) was further separated by medium pressure CC on silica gel (Katayama Chemical, Osaka, K230 53 g) with $CHCl_3$ - MeOH (30 : 1) to give garcinianin (**1**) 423 mg and GB-1 (**2**) 1243 mg.

Garcinianin (1)

Pale brownish powder. $[\alpha]_D$ 0 ° (*c* 0.1, MeOH; cell length 100 mm). HRFABms, *m/z* 557.1119 $[M+H]^+$ ($C_{30}H_{21}O_{11}$ requires: 557.1084). Uv (MeOH) λ_{max} nm (ε): 275 (16700), 287 (17600), 330sh (9300). Ir (film) ν 3500 br., 1702, 1642 cm^{-1}. ^{1}H Nmr, δ_H (150 °C) 4.89 (1H, d, *J*=12.2 Hz, H-3), 5.73 (1H, d, *J*=12.2 Hz, H-2), 5.98 (1H, near s, H-8), 6.01 (1H, near s, H-6), 6.21 (1H, s, H-6"), 6.54 (2H, d, *J*=8.8 Hz, H-3', 5'), 6.81 (2H, d, *J*=8.8 Hz, H-3"', 5"'), 7.05 (2H, d, *J*=8.8 Hz, H-2', 6'), 7.84 (2H, d, *J*=8.8 Hz, H-2"', 6"'). δ_H (25 °C) 4.92 (d, *J*=12.1 Hz), 4.98 (d, *J*=12.1 Hz) [H-3], 5.54 (d, *J*=12.1 Hz), 5.68 (d, *J*=12.1 Hz) [H-2], 5.94 (near s), 5.96 (near s) [H-8], 5.94 (near s), 6.04 (near s)

[H-6], 6.06 (s), 6.23 (s) [H-6"], 6.35 (d, J=8.8 Hz), 6.58 (d, J=8.8 Hz) [H-3', H-5'], 6.62 (d, J=8.8 Hz), 6.95 (d, J=8.8 Hz) [H-3''', H-5'''], 7.05 (d, J=8.8 Hz), 7.08 (d, J=8.8 Hz) [H-2', 6'], 7.75 (d, J=8.8 Hz), 8.02 (d, J=8.8 Hz) [H-2''', H-6'''], 12.20 (s), 12.28 (s) [OH-5], 12.49 (s), 12.57 (s) [OH-5"]. ^{13}C Nmr : see Table 1.

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