A STUDY OF BIFLAVANONES FROM THE STEMS OF *GARCINIA KOLA* (GUTTIFERAE)

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Abstract —— A new biflavanone (**1**) named (+)-GB-1b was isolated together with five known biflavonoids from the stems of *Garcinia kola* Heckel (Guttiferae) collected in Nigeria. The structure was characterized by analyses of the spectral data. The stereostructures of (-)-GB-1a, (+)-GB-1b and (-)-GB-2a are also discussed on the basis of chemical and spectroscopic evidence. Furthermore, 3,8"-biflavanones were successfully converted to 3,6"-biflavanone under thermal conditions.

Many biflavonoids were reported as the constituents of the seeds and stem barks of *Garcinia kola* Heckel (Guttiferae) collected in Nigeria, Ghana and Zaire.[1,2] In the previous papers, we also reported the isolation of arylbenzofurans, arylbenzopyran, biflavonoid and chromanols from the roots and seeds of *Garcinia kola* Heckel (Guttiferae) collected in Nigeria.[3-6] Our further study on the constituents of the above plant led to the isolation of a new biflavanone (**1**) together with five known biflavonoids (**2** - **6**). In this paper, we describe the isolation and structural characterization of the biflavonoids (**1** - **6**) from the stems of *G. kola*, and a thermal conversion of 3,8"-biflavanones to 3,6"-biflavanone.

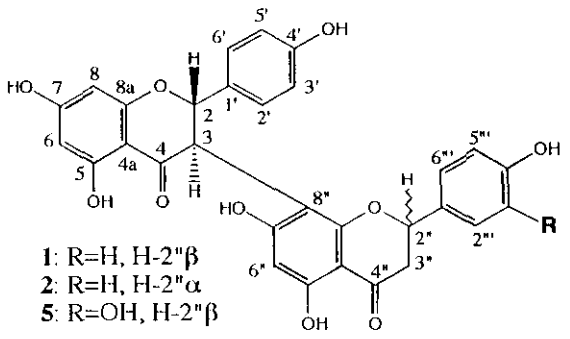
Isolation The methanol extract of the stems of *Garcinia kola* collected in Nigeria was successively partitioned between water and hexane, chloroform, ethyl acetate and butanol to give the corresponding solubles, respectively. The ethyl acetate soluble fraction was separated by medium-pressure column chromatography (MPCC) on silica gel using a mixture of chloroform and methanol (50 : 1) into five fractions. Each fraction was subjected to further separations by a combination of column chromatography on silica gel gel using a mixture of chloroform and methanol, MPCC on reversed-phase silica gel (C-8)

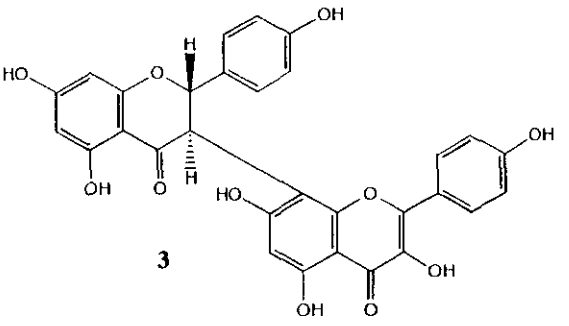
using a mixture of methanol and water and recycled HPLC on reversed-phase silica gel (C-8) using a mixture of methanol and water to give a new biflavanone named (+)-GB-1b (**1**) together with five known biflavonoids of (-)-GB-1a (**2**),[1] garcinianin (**3**),[5] amentoflavone (**4**),[7] (-)-GB-2a (**5**)[2] and 3,8"-biapigenin (**6**).[9] These known compounds (**2** - **6**) were respectively identified by comparison with the spectral data described in the literature.[1,2,5,7,9]

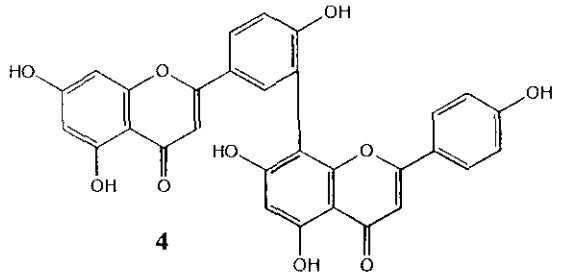
Table 1. ^{1}H and ^{13}C NMR data of (+)-GB-1b (**1**) and (-)-GB-1a (**2**)

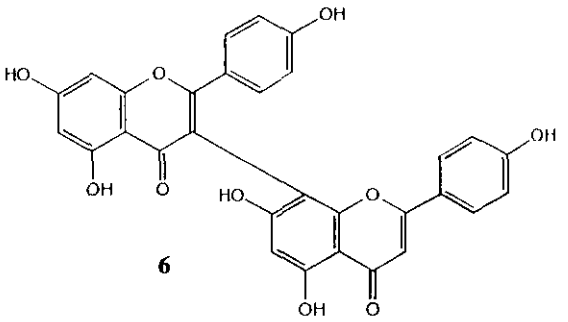
	(+)-GB-1b (**1**) [a,b]			(-)-GB-1a (**2**)[a,c]		
	^{13}C		^{1}H	^{13}C		^{1}H
2	81.0	(d)	5.58 (d, *J*=12.0 Hz)	81.3	(d)	5.57 (d, *J*=12.0 Hz)
3	47.4	(d)	4.47 (d, *J*=12.0 Hz)	47.3	(d)	4.58 (d, *J*=12.0 Hz)
4	196.6	(s)		195.5	(s)	
5	163.4	(s)		163.4	(s)	
6	96.0	(d)	5.88 (br s)	95.8	(d)	5.91 (br s)
7	166.2	(s)		166.0	(s)	
8	95.0	(d)	5.83 (br s)	95.2	(d)	5.84 (br s)
4a	101.3	(s)		101.0	(s)	
8a	162.8	(s)		162.4	(s)	
1'	128.4	(s)		128.9	(s)	
2', 6'	128.0	(d)	6.95 (d, *J*=8.3 Hz)	127.5	(d)	7.08 (d, *J*=8.0 Hz)
3', 5'	114.5	(d)	6.59 (d, *J*=8.3 Hz)	114.4	(d)	6.67 (d, *J*=8.0 Hz)
4'	157.4	(s)		157.3	(s)	
2"	79.2	(d)	4.55 (d, *J*=13.4 Hz)	78.0	(d)	5.42 (d, *J*=12.2 Hz)
3"	42.2	(t)	3.08 (dd, *J*=13.4, 16.8 Hz)	42.6	(t)	3.10 (dd, *J*=12.2, 16.1 Hz)
			2.54 (d, *J*=16.8 Hz)			2.68 (d, *J*=16.1 Hz)
4"	196.6	(s)		196.2	(s)	
5"	162.3	(s)		162.4	(s)	
6"	96.0	(d)	5.77 (s)	94.7	(d)	5.86 (s)
7"	164.6	(s)		164.6	(s)	
8"	101.9	(s)		101.2	(d)	
4"a	101.3	(s)		101.0	(s)	
8"a	162.8	(s)		161.9	(s)	
1"'	128.7	(s)		128.9	(s)	
2"', 6"'	128.0	(d)	7.25 (d, *J*=8.3 Hz)	127.5	(d)	7.16 (d, *J*=8.0 Hz)
3"', 5"'	115.1	(d)	6.81 (d, *J*=8.3 Hz)	114.9	(d)	6.62 (d, *J*=8.0 Hz)
4"'	157.5	(s)		157.3	(s)	

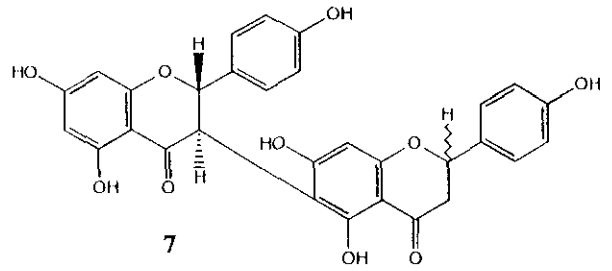
[a] Assignments are based on COSY, HMQC and HMBC.
[b] Dimethyl sulfoxide-d_6 at 25 °C.
[c] Dimethyl sulfoxide-d_6 at 100 °C.











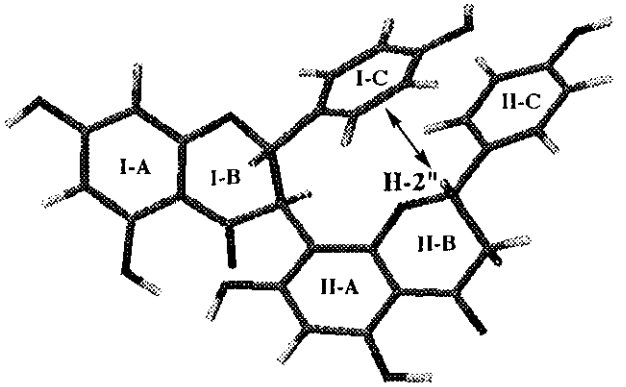


Figure 1. Stereostructure of (+)-GB-1b (**1**)

Structure of (+)-GB-1b Compound (**1**) named (+)-GB-1b, $[\alpha]_D$ +8.2° (*c* 0.22, MeOH) was found to have the molecular formula $C_{30}H_{22}O_{10}$ determined by high-resolution FABMS, which was the same molecular formula as that of (-)-GB-1a. (+)-GB-1b (**1**) was very similar to (-)- GB-1a in not only MS, IR and UV spectra but also ^1H and ^{13}C NMR spectra as shown in Table 1. (+)-GB-1b (**1**) has four sets of an *ortho*-coupled aromatic, one set of a *meta*-coupled aromatic and non-coupled aromatic hydrogens and the corresponding carbons. The presence of the *trans*-AB type signals at δ_{H-2} 5.58 ppm and δ_{H-3} 4.47 ppm having a coupling constant *J*=12.0 Hz and of the ABB' type signals at $\delta_{H-2''}$ 4.55 ppm (d, *J*=13.4 Hz), $\delta_{H-3''}$ 3.08 ppm (dd, *J*=13.4, 16.8 Hz) and $\delta_{H-3''}$ 2.54 ppm (d, *J*=16.8 Hz) showed (+)-GB-1b (**1**) to be 3,6"- or 3,8"-binaringenin. The HMBC spectrum showed the following cross peaks: between C-2 and H-2'(6'), between C-2" and H-2'"(6'"), between C-6" and $C_{5''}$ -OH, between C-8" and H-6" and between C-8" and H-3. These cross peaks indicated (+)-GB-1b (**1**) to be 3,8"-binaringenin and to be a stereoisomer of the known compound, (-)-GB-1a (**2**) $[\alpha]_D$ -28.1° (*c* 0.22, MeOH) whose stereochemistry at the C-2/C-3 positions has the *trans* geometry and whose stereochemistry at the C-2" position remains still unknown. The structural relation between (-)-GB-1a and (+)-GB-1b was chemically confirmed as follows.

(-)-GB-1a (**2**) ($[\alpha]_D$ -28.1°) was heated in DMSO at 110 °C for 2 h to give a mixture of (-)-GB-1a (**2**) $[\alpha]_D$ -3.5° (*c* 0.26, MeOH), (+)-GB-1b (**1**) $[\alpha]_D$ +2.3° (*c* 0.29, MeOH) and a new compound (**7**) $[\alpha]_D$ -6.0° (*c* 0.39, MeOH), which were isolated by recycled HPLC in 18, 20 and 27 % yields, respectively. (+)-GB-1b (**1**) ($[\alpha]_D$ +8.2°) was also heated under the same conditions to give the same mixture except for the optical rotation values of **1** and **2**. Both **1** and **2** obtained by the thermal reaction were partially racemized. However, compound (**7**) showed no change to **1** and/or **2** under the same reaction conditions.

Structure of Compound (7) The structure of compound (**7**) newly produced in the thermal reactions of GBs-1a (**2**) and/or -1b (**1**) was determined as follows. Compound (**7**) has the same partial structures as well as the molecular formula $C_{30}H_{22}O_{10}$ as those of GB-1a (**2**) and GB-1b (**1**). Compound (**7**) has four sets of an *ortho*-coupled aromatic at $\delta_{H-2',6'}$ 7.19 ppm (d, *J*=8.5 Hz), $\delta_{H-3',5'}$ 6.64 ppm (d, *J*=8.5 Hz), $\delta_{H-2''',6'''}$ 7.27 ppm (d, *J*=8.2 Hz), $\delta_{H-3''',5'''}$ 6.76 ppm (d, *J*=8.2 Hz), one set of *meta*-coupled aromatic hydrogens at δ_{H-6} 5.90 ppm (d, *J*=2.2 Hz) and δ_{H-8} 5.87 ppm (d, *J*=2.2 Hz), and a non-coupled aromatic hydrogen at $\delta_{H-8''}$ 5.78 ppm (s), *trans*-AB type signals at δ_{H-2} 5.76 ppm (d, *J*=12.4 Hz) and δ_{H-3} 4.69 ppm (d, *J*=12.4 Hz), and ABB' type signals at $\delta_{H-2''}$ 5.38 ppm (d, *J*=12.2 Hz), $\delta_{H-3''}$ 3.14 ppm (dd, *J*=12.2, 16.1 Hz) and $\delta_{H-3''}$ 2.59 ppm (d, *J*=16.1 Hz). From these data, compound (**7**) seems to be a regioisomer of GB-1a (**2**) and GB-1b (**1**). The HMBC spectrum of **7** showed the following cross peaks: between C-2 and H-2'(6'), between C-5 and C_5 -OH, between C-5" and H-3, between C-5" and $C_{5''}$ -OH, between C-6" and H-3 and between C-7" and H-3. These data indicated compound (**7**) to be 3,6"-binaringenin. The stereochemistry at the position of C-2" of compound (**7**) remains still unknown. Compound (**7**) showed further no change under the thermal reaction condition (140 °C, 2 h). This is the first report of the thermal conversion from 3,8"-biflavanone to 3,6"-biflavanone.

Stereochemistry of (-)-GB-1a, (+)-GB-1b and (-)-GB-2a In the ^{1}H NMR, the chemical shift value (δ 4.55 ppm) at the H-2" position of (+)-GB-1b (**1**) was very different from that of (-)-GB-1a (**2**; δ 5.54 and 5.36 ppm at 25 °C, δ 5.42 ppm at 100 °C).[10] The high field shift of the H-2" owing to an anisotropic effect indicated that H-2" was situated below a benzene ring (I-C), as shown in Figure 1. These data, together with the study using the Dreiding stereomodel, suggested the stereochemistry of GB-1b to be 2R,3R,2"R or 2S,3S,2"S. This conclusion also led the stereochemistry of GB-1a (**2**) to 2R,3R,2"S or 2S,3S,2"R. Furthermore, the chemical shift value (δ 4.66 ppm) at the H"-2 position of (-)-GB-2a (**5**) suggested the stereochemistry of **5** to be 2R,3R,2"R or 2S,3S,2"S. The absolute configurations of **1**, **2** and **5** remain still unknown.

EXPERIMENTAL

Optical rotations were recorded on a JASCO P-1020 polarimeter at 25 °C, using a 100-mm length quartz cell, unless otherwise indicated. UV spectra were taken in MeOH with a JASCO V-560 spectrophotometer. IR spectra were taken on a JASCO FT/IR-410 spectrophotometer. MS spectra were obtained under EI conditions with a Hitachi M-80 spectrometer and under FAB conditions with a JEOL HX-110 spectrometer. ^{1}H-, ^{13}C-, two-dimensional (2D) NMR and difference NOE spectra were measured with JEOL α-400 and α-600 spectrometers in DMSO-d_6, unless otherwise indicated.

Isolation Young stems of *Garcinia kola* (2.3 kg) collected in Nigeria were extracted with MeOH (8 L x 3) at rt for 3 days to yield an extract (105 g). The MeOH extract was subjected to the fractionization with hexane (2.2 g), $CHCl_3$ (3.8 g), EtOAc (7.8 g) and BuOH (7.8 g), successively. The EtOAc soluble fraction was separated by MPCC on SiO_2 (Fuji Silysia Co. Ltd., BW-820 MH, 140 g) using a mixture of $CHCl_3$ and MeOH (50 : 1, flow rate 3.0 mL/min) to give 4 fractions ; 306 mg, 690 mg, 202 mg and 176 mg and then using a mixture of $CHCl_3$ and MeOH (9 : 1) to give a fraction (5.25 g).

1) The second fraction (690 mg) was column chromatographed (CC) on SiO_2 (Fuji Silysia Co. Ltd., BW-820 MH, 25 g) with $CHCl_3$ and MeOH (50 : 1) to give 3 fractions (32 mg, 450 mg, 37 mg). The first fraction (32 mg) was separated by recycled HPLC [YMC Co. Ltd., YMC-Pack C8 {φ20 x 250 cm, MeOH - H_2O (7 : 3), flow rate: 3.0 mL/min}] to give (-)-GB-1a (**2**) (14.1 mg) and (+)-GB-1b (**1**) (9.6 mg). The third fraction (37 mg) from CC was separated by recycled HPLC [YMC Co. Ltd., YMC-Pack C8 {φ20 x 250 cm, MeOH - H_2O (8 : 2), flow rate: 3.0 mL/min}] to give garcinianin (**3**) (7 mg).

2) The third fraction (202 mg) was subjected to CC on SiO_2 (Fuji Silysia Co. Ltd., BW-820 MH, 25 g) with $CHCl_3$ and MeOH (50 : 1) to give 2 fractions (156 mg, 30 mg). The first fraction (156 mg) was separated by reversed phase MPCC [Nomura Chemical Co. Ltd., Develosil Lop C8 {φ30 x 300 cm, MeOH - H_2O (6 : 4), flow rate: 3.0 mL/min}] to give three fractions (40 mg, 68 mg, 17 mg). The third fraction (17 mg) was separated by recycled HPLC [YMC Co. Ltd., YMC-Pack C8 {φ20 x 250 cm, MeOH - H_2O (8 : 2), flow rate: 3.0 mL/min}] to give amentoflavone (**4**) (2.3 mg).

3) The fourth fraction (176 mg) was separated by reversed phase MPCC [Nomura Chemical Co. Ltd., Develosil Lop C8 {φ30 x 300 cm, MeOH - H_2O (6 : 4), flow rate: 3.0 mL/min}] to give six fractions (16 mg, 17 mg, 82 mg, 3 mg, 22 mg, 7 mg). The second and fifth fractions (17 mg and 22 mg) were separated by recycled HPLC [YMC Co. Ltd., YMC-Pack C8 {φ20 x 250 cm, MeOH - H_2O (8 : 2), flow rate: 3.0 mL/min}] to give GB-2a (**5**) (7 mg) and 3,8"-biapigenin (**6**) (3.3 mg), respectively.

Characterization

(+)-GB-1b (**1**): $[\alpha]_D$ +8.2° (*c* 0.22, MeOH); UV (MeOH), λ 338 nm (log ε 3.8), 292 (4.4), 250 (3.9), 225 (4.9); IR (KBr), ν 3430 br, 1634 cm^{-1}; HRFABMS, *m/z* 543.1273 [M+H$^+$] ($C_{30}H_{23}O_{10}$ requires: 543.1291); ^{1}H NMR see Table 1; ^{13}C NMR see Table 1.

(-)-GB-1a (**2**): $[\alpha]_D$ -28.1° (*c* 0.22, MeOH); UV (MeOH), λ 332 nm (log ε 3.7), 292 (4.3), 253 (3.8), 227 (4.5); IR (KBr), ν 3430 br, 1630 cm^{-1}; HRFABMS, *m/z* 543.1276 [M+H$^+$] ($C_{30}H_{23}O_{10}$ requires: 543.1291); ^{1}H NMR at 25 °C, δ 5.67 (1H, d, *J*=12.0 Hz, H-2), 4.50 (1H, d, *J*=12.0 Hz, H-3), 5.89 (1H, br s, H-6), 5.84 (1H, br s, H-8), 7.12 (2H, d, *J*=8.0 Hz, H-2',6'), 6.79 (2H, d, *J*=8.0 Hz, H-3',5'), 5.54 (1H, d, *J*=12.7 Hz, H-2"), 2.92 (1H, dd, *J*=12.7, 16.8 Hz, H-3"), 2.72 (1H, d, *J*=16.8 Hz, H-3"), 5.76 (1H, s, H-6"), 7.09 (2H, d, *J*=8.0 Hz, H-2"',6"'), 6.63 (2H, d, *J*=8.0 Hz, H=3"',5"'), 5.37 (1H, d, *J*=12.0 Hz, H-2), 4.65 (1H, d, *J*=12.0 Hz, H-3), 5.91 (1H, br s, H-6), 5.89 (1H, br s, H-8), 7.20 (2H, d, *J*=8.0 Hz, H-2',6'), 6.83 (2H, d, *J*=8.0 Hz, H-3',5'), 5.36 (1H, d, *J*=12.7 Hz, H-2"), 2.90 (1H, dd, *J*=12.7, 16.8 Hz, H-3"), 2.72 (1H, d, *J*=16.8 Hz, H-3"), 5.80 (1H, s, H-6"), 7.09 (2H, d, *J*=8.0 Hz, H-2"',6"'), 6.63 (2H, d, *J*=8.0 Hz, H=3"',5"'); ^{13}C NMR at 25 °C, δ 81.3 (d, C-2), 47.4 (d, C-3), 196.1 (s, C-4), 159.7 (s, C-5), 96.1 (d, C-6), 164.6 (s, C-7), 95.0 (d, C-8), 101.1 (s, C-4a), 162.7 (s, C-8a), 128.0 (s, C-1'), 126.7 (d, C-2', 6'), 114.7 (s, C-3', 5'), 157.1 (s, C-4'), 78.2 (d, C-2"), 42.7 (t, C-3"), 196.6 (s, C-4"), 163.6 (s, C-5"), 95.0 (d, C-6"), 166.4 (s, C-7"), 101.4 (s, C-8"), 101.3 (s, C-4"a), 162.0 (s, C-8"a), 129.0 (s, C-1"'), 128.9 (d, C-2"',6"'), 115.0 (s, C-3"',5"'), 157.7 (s, C-4"'), 81.8 (d, C-2), 47.4 (d, C-3), 196.1 (s, C-4), 160.7 (s, C-5), 96.1 (d, C-6), 165.0 (s, C-7), 95.0 (d, C-8), 101.1 (s, C-4a), 162.8 (s, C-8a), 128.0 (s, C-1'), 127.8 (d, C-2', 6'), 114.7 (s, C-3', 5'), 157.5 (s, C-4'), 78.4 (d, C-2"), 43.1 (t, C-3"), 196.8 (s, C-4"), 163.8 (s, C-5"), 95.0 (d, C-6"), 166.4 (s, C-7"), 101.6 (s, C-8"), 101.3 (s, C-4"a), 162.5 (s, C-8"a), 129.1 (s, C-1"'), 128.9 (d, C-2"',6"'), 115.2 (s, C-3"',5"'), 157.7 (s, C-4"'); ^{1}H NMR at 100 °C see Table 1; ^{13}C NMR at 100 °C see Table 1.

Amentoflavone (**4**): $[\alpha]_D$ -3.4° (*c* 0.11, MeOH); UV (MeOH), λ 329 nm (log ε 2.8), 291 (2.8), 227 (3.0), 204 (3.2); IR (KBr), ν 3420 br, 1643 cm^{-1}; FABMS, *m/z* 539 [M+H$^+$]; ^{1}H NMR, δ 6.72 (1H, s, H-3), 6.50 (1H, d, *J*=2.0 Hz, H-6), 6.23 (1H, d, *J*=2.0 Hz, H-8), 8.14 (1H, d, *J*=2.0 Hz, H-2'), 7.22 (1H, d, *J*=8.5 Hz, H-5'), 8.02 (1H, dd, *J*=8.5, 2.0 Hz, H-6'), 6.65 (1H, s, H-3"), 6.42 (1H, s, H-6"), 7.66 (2H, d, *J*=8.5 Hz, H-2"', 6"'), 6.81 (2H, d, *J*=8.5 Hz, H-3"', 5"'); ^{13}C NMR, δ 164.1 (s, C-2), 102.8 (d, C-3), 181.7 (s, C-4), 161.4 (s, C-5), 98.8 (d, C-6), 164.0 (s, C-7), 94.0 (d, C-8), 103.7 (s, C-4a), 157.4 (s, C-8a), 121.5 (s, C-1'), 128.2 (d, C-2'), 121.5 (s, C-3'), 160.5 (s, C-4'), 116.8 (d, C-5'), 131.4 (d, C-6'), 164.1 (s, C-2"), 102.6 (d, C-3"), 182.0 (s, C-4"), 160.9 (s, C-5"), 99.8 (d, C-6"), 161.4 (s, C-

7"), 103.7 (s, C-8"), 103.7 (s, C-4"a), 154.6 (s, C-8"a), 121.5 (s, C-1'"), 128.2 (d, C-2'", 6'"), 115.7 (d, C-3'", 5'"), 160.9 (s, C-4'").

GB-2a (**5**): $[\alpha]_D$ -10.5° (*c* 0.35, MeOH); UV (MeOH), λ 341 nm (log ε 2.7), 292 (3.4), 225 (3.5), 202 (3.7); IR (KBr), ν 3420 br, 1639 cm^{-1}; FABMS, *m/z* 559 $[M+H^+]$; ^1H NMR, δ 5.62 (1H, d, *J*=12.0 Hz, H-2), 4.66 (1H, d, *J*=12.0 Hz, H-3), 5.83 (1H, br s, H-6), 5.88 (1H, br s, H-8), 7.18 (2H, d, *J*=8.3 Hz, H-2',6'), 6.62 (2H, d, *J*=8.3 Hz, H-3',5'), 4.66 (1H, d, *J*=12.2 Hz, H-2"), 3.20 (1H, dd, *J*=12.2, 16.1 Hz, H-3"), 2.68 (1H, d, *J*=16.1 Hz, H-3"), 5.85 (1H, s, H-6"), 6.89 (1H, d, *J*=3.0 Hz, H-2'"), 6.66 (1H, d, *J*=8.3 Hz, H=5'"), 7.03 (1H, dd, *J*=8.3, 3.0 Hz, H-6'"); ^{13}C NMR, δ 81.1 (d, C-2), 47.3 (d, C-3), 196.2 (s, C-4), 165.2 (s, C-5), 95.8 (d, C-6), 166.0 (s, C-7), 95.8 (d, C-8), 101.3 (s, C-4a), 165.2 (s, C-8a), 128.5 (s, C-1'), 128.5 (d, C-2', 6'), 114.4 (s, C-3', 5'), 157.2 (s, C-4'), 78.3 (d, C-2"), 41.9 (t, C-3"), 199.6 (s, C-4"), 165.2 (s, C-5"), 94.7 (d, C-6"), 166.0 (s, C-7"), 101.3 (s, C-8"), 101.3 (s, C-4"a), 163.4 (s, C-8"a), 127.8 (s, C-1'"), 114.5 (d, C-2'"), 144.9 (s, C-3'"), 145.2 (s, C-4'"), 115.2 (d, C-5'"), 128.2 (s, C-6'").

3,8"-Biapigenin (**6**): UV (MeOH), λ 329 nm (log ε 3.1), 271 (3.3), 202 (3.5); IR (KBr), ν 3220 br, 1639 cm^{-1}; FABMS, *m/z* 539 $[M+H^+]$; ^1H NMR, δ 6.24 (1H, d, *J*=2.0 Hz, H-6), 6.50 (1H, d, *J*=2.0 Hz, H-8), 7.34 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.67 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.72 (1H, s, H-3"), 6.23 (1H, s, H-6"), 7.66 (2H, d, *J*=8.5 Hz, H-2'", 6'"), 6.81 (2H, d, *J*=8.5 Hz, H-3'", 5'"); ^{13}C NMR, δ 163.4 (s, C-2), 110.2 (s, C-3), 191.8 (s, C-4), 161.1 (s, C-5), 99.0 (d, C-6), 163.3 (s, C-7), 93.8 (d, C-8), 102.8 (s, C-4a), 157.4 (s, C-8a), 122.9 (s, C-1'), 129.7 (d, C-2', 6'), 115.9 (s, C-3', 5'), 161.5 (s, C-4'), 163.4 (s, C-2"), 102.9 (d, C-3"), 180.5 (s, C-4"), 159.8 (s, C-5"), 99.3 (d, C-6"), 163.3 (s, C-7"), 99.3 (s, C-8"), 102.9 (s, C-4"a), 154.9 (s, C-8"a), 121.4 (s, C-1'"), 128.0 (d, C-2'", 6'"), 115.2 (d, C-3'", 5'"), 161.1 (s, C-4'").

Thermal reaction

Thermal reaction of (-)-GB-1a A solution of (-)-GB-1a (**2**) (20 mg) in DMSO (0.5 mL) was heated at 110 ℃ for 2 h. After concentration *in vacuo*, the residue was separated by recycled HPLC [Nacalai Tesque Co. Ltd., Cosmosil C8 {φ20 x 250 cm, MeOH - H_2O (7 : 3), flow rate: 3.0 mL/min}] to give (-)-GB-1a (**2**) (3.7 mg (18.5 %); $[\alpha]_D$ -3.5° (*c* 0.26, MeOH)), (+)-GB-1b (**1**) (4.0 mg (20.0 %); $[\alpha]_D$ +2.3° (*c* 0.29, MeOH)), and **7** (5.5 mg (27.5 %); $[\alpha]_D$ -6.0° (*c* 0.39, MeOH)).

Thermal reaction of (+)-GB-1b A solution of (+)-GB-1b (**1**) (19 mg) in DMSO (0.5 mL) was heated at 110 °C for 2 h. After concentration *in vacuo*, the residue was separated by recycled HPLC [Nacalai Tesque Co. Ltd., Cosmosil C8 {φ20 x 250 cm, MeOH - H_2O (7 : 3), flow rate: 3.0 mL/min}] to give (-)-GB-1a (**2**) (3.1 mg (16.3 %); $[\alpha]_D$ -1.5° (*c* 0.16, MeOH)), (+)-GB-1b (**1**) (2.6 mg (13.7 %); $[\alpha]_D$ +3.0° (*c* 0.10, MeOH)), and **7** (5.5 mg (28.9 %); $[\alpha]_D$ -2.3° (*c* 0.28, MeOH)).

Compound (7) : UV(MeOH), λ 334 nm (log ε 3.7), 292 (4.3), 253 (3.5), 226 (4.5); IR (KBr), ν 3400 br, 1634 cm^{-1}; FABMS, *m/z* 543 $[M+H^+]$; ^1H NMR, δ 5.76 (1H, d, *J*=12.4 Hz, H-2), 4.69 (1H, d, *J*=12.4 Hz, H-3), 5.90 (1H, d, *J*=2.2 Hz, H-6), 5.87 (1H, d, *J*=2.2 Hz, H-8), 7.19 (2H, d, *J*=8.5 Hz, H-2',

6'), 6.64 (2H, d, *J*=8.5 Hz, H-3', 5'), 5.38 (1H, d, *J*=12.2 Hz, H-2"), 3.14 (1H, dd, *J*=12.2, 16.1 Hz, H-3"), 2.59 (1H, d, *J*=16.1 Hz, H-3"), 5.78 (1H, s, H-8"), 7.27 (2H, d, *J*=8.2 Hz, H-2"', 6"'), 6.76 (2H, d, *J*=8.2 Hz, H-3"', 5"'), 12.60 (2H, s, $C_{5,5''}$-OH); ^{13}C NMR, δ 81.1 (d, C-2), 46.4 (d, C-3), 196.9 (s, C-4), 163.6 (s, C-5), 96.0 (d, C-6), 166.4 (s, C-7), 95.0 (d, C-8), 101.3 (s, C-4a), 162.9 (s, C-8a), 128.0 (s, C-1'), 129.0 (d, C-2', 6'), 114.8 (d, C-3', 5'), 157.6 (s, C-4'), 78.6 (d, C-2"), 48.7 (t, C-3"), 196.6 (s, C-4"), 163.6 (s, C-5"), 101.7 (d, C-6"), 164.4 (s, C-7"), 96.0 (s, C-8"), 106.3 (s, C-4"a), 161.6 (s, C-8"a), 128.8 (s, C-1"'), 128.4 (d, C-2"', 6"'), 115.3 (d, C-3"', 5"'), 157.7 (s, C-4"').

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10. (-)-GB-1a (**2**) has mainly two conformers at 25 °C in dimethyl sulfoxide-d_6; ^{1}H and ^{13}C NMR spectral data at 25 °C were shown in the EXPERIMENTAL. The assignments for each conformer were mainly based on 2D-NMR spectra.

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