Revised Structures of Gambiriins A1, A2, B1, and B2, Chalcane-Flavan Dimers from Gambir (*Uncaria gambir* Extract)

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Gambir, the aqueous extract from *Uncaria gambir* (Rubiaceae), has been used as an astringent medicine in Asian countries. Investigation of the constituents in the extract led to the isolation of four chalcane-flavan dimers, gambiriin A1 (6), A2 (7), B1 (8), and B2 (9), in addition to (+)-catechin (1), (+)-epicatechin (2), and dimeric proanthocyanidins, procyanidin B1 (3), procyanidin B3 (4), and gambiriin C (5). The spectroscopic and chemical data obtained in the present study indicated that their previously proposed structures 6a, 7a, 8a, and 9a should be revised to 6, 7, 8, and 9, respectively.

Key words gambir; *Uncaria gambir*; chalcane-flavan dimer; catechin; gambiriin

Gambir, an official pharmacopoeic medicine in Japan, is the aqueous extract of the leaves and young twigs of *Uncaria gambir* Roxb. (Rubiaceae).[2)] The extract has been used for the treatment of diarrhea and sore throat as an astringent medicine in Southeast Asia,[3)] and also for hoarseness, as an ingredient of the kampo medicine "Kyoseihatekigan."

Flavan monomers, (+)-catechin (**1**) and (+)-epicatechin (**2**),[4)] several other dimeric compounds[5)] related to **1**, as well as alkaloids,[6,7)] have been reported to be the constituents. Our investigation on the polyphenolic contents of a manufactured gambir product led to the isolation of **1**, **2**, and seven dimeric flavans **3**—**9**. Although the seven dimeric flavans were known compounds, the present study revealed that the stereostructures of four of the chalcane-flavan dimers should be revised to **6**—**9**. Their ^{1}H- and ^{13}C-NMR spectral assignments are also shown here, since those were not reported in detail previously.[5,8)]

Results and Discussion

A manufactured product of gambir purchased in Japan was extracted with MeOH, and the extract was fractionated by column chromatography on Dia-ion HP-20 using aqueous MeOH. (+)-Catechin (**1**) was crystallized from the eluate with 20% MeOH, and the mother liquor was subjected to column chromatography on a Toyopearl HW-40 to give a fraction containing both monomeric and dimeric flavans. The fraction was further chromatographed on octadecylsilyl (ODS) silica gel, Sephadex LH-20, and MCI-gel CHP-20P columns to give (+)-catechin (**1**), (+)-epicatechin (**2**), procyanidin B1 (**3**),[9,10)] procyanidin B3 (**4**),[11)] gambiriin C (**5**),[5)] and gambiriin A1 (**6**). The eluate, with 40% MeOH from the column of Dia-ion HP-20, was separated in an analogous way to give gambiriin A2 (**7**), gambiriin B1 (**8**), and gambiriin B2 (**9**).

Gambiriin A1 (**6**) was obtained as an amorphous powder. The dimeric molecular formula, $C_{30}H_{28}O_{12}$, was indicated by the $[M+H]^+$ ion peak at *m*/*z* 581 in the electrospray-ionization (ESI)-MS. The ^{1}H-NMR spectrum showed signals due to A- and A′-ring protons [δ: 5.95 (2H, s, H-3$_U$, H-5$_U$), 6.10 (1H, s, H-6$_L$) (U and L mean the upper and lower units, respectively.)], and two sets of ABX signals due to B- and B′-ring protons [δ: 6.65 (1H, d, J=8.5 Hz, H-5′$_U$), 6.68 (2H, m, H-6′$_L$, H-6′$_U$), 6.71 (1H, d, J=8.5 Hz, H-5′$_L$), 6.83 (1H, d, J=2.0 Hz, H-2′$_U$), 6.87 (1H, d, J=2.0 Hz, H-2′$_L$)] in the aromatic region (see formula **6**). Discrimination of the B- and B′-ring protons was achieved based on the long range ^{1}H–^{1}H correlations, H-α_U/H-2′$_U$ and H-α_U/H-6′$_U$. The spectrum also showed two sets of the methine–methine–methylene connectivities in the aliphatic region. H-2$_L$ and H-3$_L$ in one set [δ: 4.63 (H-2$_L$), 3.98 (H-3$_L$), 2.61 (H-4a$_L$), 2.95 (H-4b$_L$)] showed a large coupling constant (7.5 Hz), indicating that the lower unit was 2,3-*trans* catechin. The 2,3-*trans* structure was also validated by the ^{13}C-NMR spectrum, which showed the C-2$_L$ signal at δ 82.5. The remaining set of the methine–methine–methylene protons [δ: 2.56 (H-γa$_U$), 2.95 (H-γb$_U$), 4.68 (H-β_U), 4.75 (H-α_U)] was attributable to the substituted propyl group in the chalcane structure. The molecular formula of **6**, which was indicated by the ESI-MS, also substantiates this open-chain chalcane structure.

The rotating frame nuclear Overhauser effect spectroscopy (ROESY) of **6** showed correlations of H-α_U/H-2′$_L$ and H-β_U/H-2′$_L$, indicating the presence of an interflavonoid link between position C-8$_L$ and the upper chalcane unit (C-α_U) (Fig. 2). These correlations would not be observed if the linkage occurred at C-6$_L$. The aliphatic protons of **6** appeared as broad signals, due to the restricted rotation around the interflavonoid linkage, which confirmed the location of the linkage at C-8$_L$.

The assigned structure of **6** based on these data suggested that it is a product from (+)-catechin (**1**). If **6** is actually produced from **1**, the absolute configurations of the asymmetric carbons in the chalcane and flavan residues of **6** could be determined based on those of **1**. Therefore, an aqueous solution of (+)-catechin (**1**) was heated to ascertain whether the dimer **6** would be produced. Compound **6** was isolated from the reaction mixture, and the 2*R*,3*S*-configurations in the lower unit and the *S*-configuration of the upper β carbon (C-β_U) were thus confirmed. Although the ^{1}H- and ^{13}C-NMR spectral data suggested the identity of **6** as gambiriin A1 (**6a**) in the literature,[5,8)] the stereochemistry of C-α_U was to be revised based on the relationship with **8**, as discussed later.

Gambiriin B1 (**8**) was obtained as a light-brown powder.

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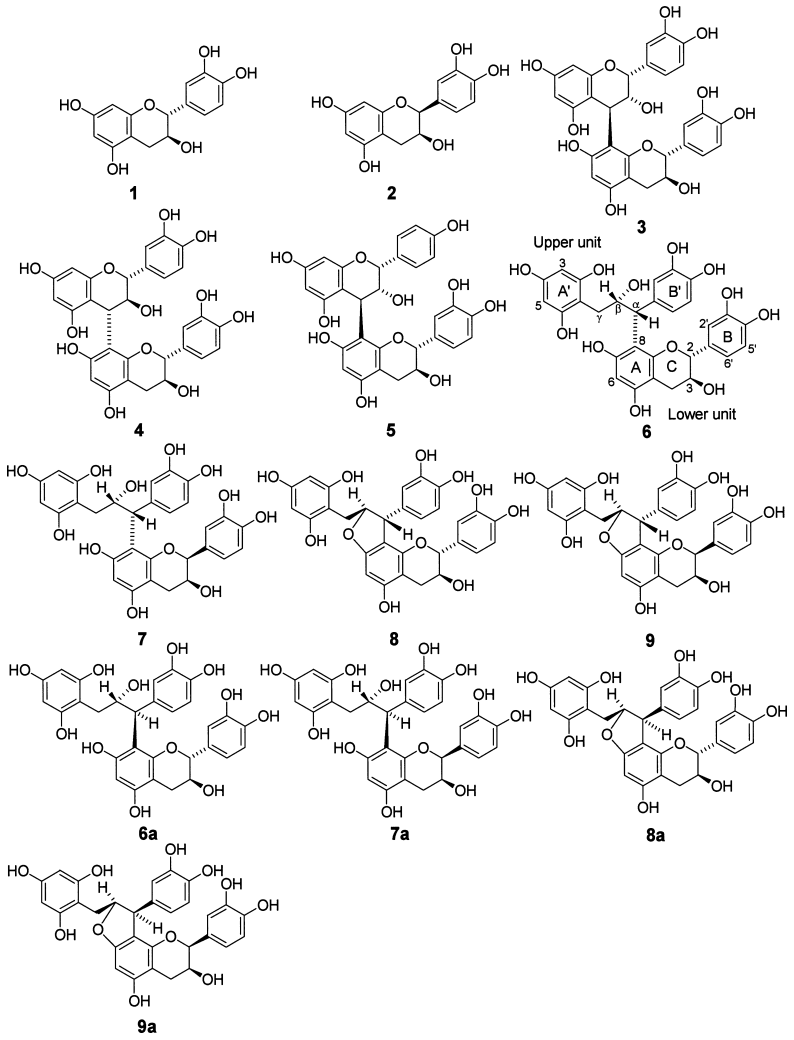

Fig. 1. Structures of Isolated Compounds, **1**—**9**, from Gambir and Previously Reported Structures of Gambiriin A1 (**6a**), A2 (**7a**), B1 (**8a**), and B2 (**9a**)

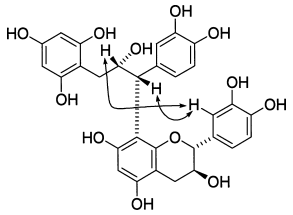

Fig. 2. Important ROE Correlations Observed for **6**

The molecular formula was determined to be $C_{30}H_{26}O_{11}$ based on the $[M+H]^+$ ion peak at *m/z* 563 in the ESI-MS, with the nominal molecular weight 18 mass unit (H_2O) less than that of gambiriin A1 (**6**). The ^{1}H-NMR spectrum showed protons of the A- and A′-rings [δ: 5.92 (2H, s, H-3_U, H-5_U), 6.01 (1H, s, H-6_L)] and two sets of ABX signals of the B- and B′-ring protons [δ: 6.20 (1H, dd, J=2.5, 8.5 Hz, H-$6'_U$), 6.24 (1H, dd, J=2.5, 8.5 Hz, H-$6'_L$), 6.41 (1H, d, J=2.5 Hz, H-$2'_U$), 6.56 (1H, d, J=8.5 Hz, H-$5'_L$), 6.58 (1H, d, J=8.5 Hz, H-$5'_U$), 6.64 (1H, d, J=2.5 Hz, H-$2'_L$)]. The spectrum also showed two sets of the methine–methine–methylene systems, which were assigned to be a C-ring [δ: 4.55 (1H, d, J=7.5 Hz, H-2_L), 3.77 (1H, ddd, J=5.5, 7.5, 8.5 Hz, H-3_L), 2.49 (1H, dd, J=8.5, 15.5 Hz, H-$4a_L$), 2.76 (1H, dd, J=5.5, 15.5 Hz, H-$4b_L$] of the catechin residue and a substituted propyl group [δ: 4.26 (1H, d, J=3.5 Hz, H-α_U), 4.79 (1H, ddd, J=3.5, 5.5, 8.5 Hz, H-β_U), 2.88 (1H, dd, J=5.5, 12.5 Hz, H-γa_U), 2.94 (1H, dd, J=8.5, 12.5 Hz, H-γb_U)] of the chalcane structure.

The ^{13}C-NMR data also showed that **8** was composed of the chalcane and catechin residues (see Experimental). The assignments of the A-ring carbons were based on the following correlations in the heteronuclear multiple-bond correlation (HMBC) spectrum: H-α_U/C-7_L, H-α_U/C-9_L, H-β_U/C-7_L, H-2_L/C-9_L, H-4_L/C-5_L, H-4_L/C-9_L, H-6_L/C-5_L, and H-6_L/C-7_L. The location of the interflavonoid linkage between C-α_U and C-8_L was indicated by the correlations H-α_U /C-9_L and H-2_L/C-9_L.

The molecular formula shown by the ESI-MS and the downfield shift of an A-ring carbon (δ 159.9; C-7_L) of **8**, relative to the corresponding carbon of **6**, suggested that **8** possessed an ether linkage at C-7_L. The HMBC correlation H-β_U/C-7_L indicated that the ether bond formed between the hydroxyl group at C-β_U and the hydroxyl group at C-7_L. The ^{1}H-NMR spectrum showed sharp signals for the aliphatic protons in spite of the location of the interflavonoid linkage at C-8_L, and the fixed conformation due to the presence of the ether linkage enabled the rationalization of this phenome-

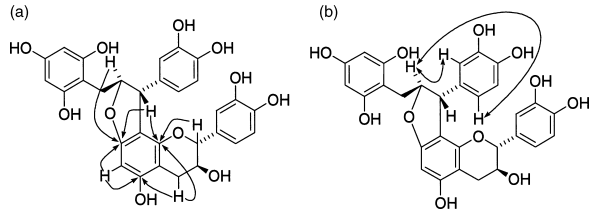

Fig. 3. Important HMBC and ROE Correlations Observed for **8**
(a) HMBC: H→C Correlation, (b) ROE.

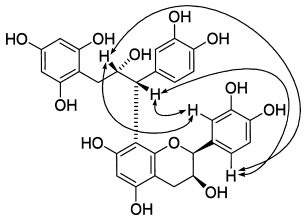

Fig. 4. Important ROE Correlations Observed for **7**

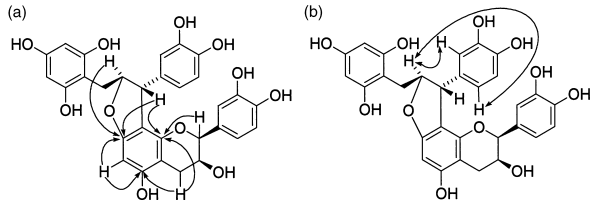

Fig. 5. Important HMBC and ROE Correlations Observed for **9**
(a) HMBC: H→C Correlation, (b) ROE.

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Compound **8** was also isolated from the product mixture formed upon the heating of (+)-catechin (**1**). The 2*R*,3*S*-configurations in the lower unit and the *S*-configuration of the upper β carbon (C-β_U) were confirmed based on a similar reasoning as for **6**.

Although the structure of gambiriin B1 (**8a**) reported previously had the *cis* relationship of H-α_U and H-β_U, the *trans* relationship between H-α_U and H-β_U was shown by the ROE correlations of H-β_U/H-2′$_U$ and H-β_U/H-6′$_U$ in the ROESY spectrum of **8**. The structure of gambiriin B1 (**8a**) was thus revised as shown by formula **8** with *R*-configuration of C-α_U.

Structures of **6** and **8** were chemically correlated by treatment of **6** with polyphosphoric acid to give gambiriin B1 (**8**). Accordingly, the stereostructure of gambiriin A1 was revised from **6a** to **6**, in which the asymmetric carbon C-α_U has the *R*-configuration.

Gambiriin A2 (**7**) was obtained as a light-brown amorphous powder. The molecular formula was determined to be $C_{30}H_{28}O_{12}$ based on the ESI-MS ion peaks at *m/z* 581 $[M+H]^+$ and 603 $[M+Na]^+$. The ^{1}H-NMR spectrum of **7**, which was similar to that of **6**, indicated that this compound was also a chalcane-flavan dimer. The differences in the ^{1}H-NMR spectra of **6** and **7** were among the signals of one set of the methine–methine–methylene systems [δ: 4.81 (1H, br s, H-2$_L$), 4.20 (1H, br s, H-3$_L$), 2.81 (1H, d, J=16.0 Hz, H-4a$_L$), 2.90 (1H, dd, J=5.0, 16.0 Hz, H-4b$_L$)] in the aliphatic region of **7**. The small coupling constant between H-2$_L$ and H-3$_L$ indicated the 2,3-*cis* configuration of the lower unit. The ^{13}C-NMR spectrum also showed the signals of the chalcane and flavan structure and the chemical shift of C-2$_L$ (δ 79.6) substantiated the 2,3-*cis* structure of the lower unit.

The position of the interflavonoid linkage on the lower unit was at C-8$_L$, since the ROESY spectrum showed the correlations between the propyl protons of the upper unit and the B′-ring protons of the lower unit (H-α_U/H-2′$_L$, H-α_U/H-6′$_L$, H-β_U/H-2′$_L$, and H-β_U/H-6′$_L$).

Compound **7** was also isolated as a product obtained upon the heating of (+)-catechin (**1**). Therefore, the epicatechin structure of the lower unit was assigned as being derived from epimerization at C-2 of **1**,[12] and the lower unit as having the 2*S*,3*S*-configuration. The production of **7** from **1** also indicated that the upper unit has the *S*-configuration at the β-carbon. Although the spectral data of **7** suggested the identity of **7** as gambiriin A2 (**7a**), the *R*-configuration of C-α_U in the structure of **7** was assigned based on the structure of gambiriin B2 (**9**), as discussed below.

Gambiriin B2 (**9**) was obtained as an amorphous powder. The molecular formula was determined to be $C_{30}H_{26}O_{11}$ based on the ion peak at *m/z* 580 $[M+NH_4]^+$ and 585 $[M+Na]^+$ in the ESI-MS. The nominal molecular weight 562 was the same as that of gambiriin B1 (**8**). Although the ^{1}H- and ^{13}C-NMR spectra of **9** were similar to those of gambiriin B1 (**8**), the differences in the ^{1}H- and ^{13}C-NMR spectra between **8** and **9** were among the ^{1}H and ^{13}C signals of the lower unit C-ring. The ^{1}H signals of the lower unit, which appeared at δ 4.71 (br s, H-2$_L$), 4.22 (m, H-3$_L$), 2.68 (dd, J=3.5, 17.0 Hz, H-4a$_L$), 2.81 (dd, J=5.0, 17.0 Hz, H-4b$_L$), indicated that the lower unit was 2,3-*cis* epicatechin in **9** instead of 2,3-*trans* catechin in **8**. The ^{13}C chemical shift of C-2$_L$ (δ 78.5) also satisfied the 2,3-*cis* structure. The HMBC correlations, H-2$_L$/C-9$_L$ and H-α_U/C-9$_L$, showed that the position of the interflavonoid linkage on the lower unit was at C-8$_L$. The formation of the ether bond between the hydroxyl group at C-β_U and the hydroxyl group at C-7$_L$ was demonstrated by the correlation H-β_U/C-7$_L$ in the HMBC spectrum.

Compound **9** was also obtained from (+)-catechin (**1**) on the heating reaction. The 2*S*,3*S*-configuration of the lower unit and the *S*-configuration of the upper β-carbon were thus confirmed. The ROESY spectrum showed the correlations of H-β_U/H-2′$_U$ and H-β_U/H-6′$_U$, which indicated that the *trans* relationship of H-α_U and H-β_U. Compound **7** was treated with polyphosphoric acid, to give **9**. The *R*-configuration of C-α_U in **7** was thus established.

The structure of gambiriin B2 reported in a previous paper, **9a**, was based on the chemical conversion of gambiriin A2 nonamethyl ether to gambiriin B2 octamethyl ether.[5] The structure for **9** assigned in the present study indicated the identity of **9** as gambiriin B2. The structure of gambiriin B2 should therefore be **9**.

Since **2** and **6**—**9** were formed from a heating reaction of **1**, these compounds might be formed in the manufacturing process of the gambir extracts, while proantocyanidin dimers **3**, **4**, and **5** are regarded to be the genuine constituents of *U. gambir* leaves.

The mechanism of the formation of **2** and **6**—**9** from **1** was considered to be that shown in Chart 1. Dimeric products **6** and **7** were produced *via* an attack of the nucleophilic A-ring of **1** (or **2**) to C-α of an intermediate **A** (formed from **1**). The β-orientation of the phenyl ring of the lower unit was attributed to conformational stability, as shown in the projec-

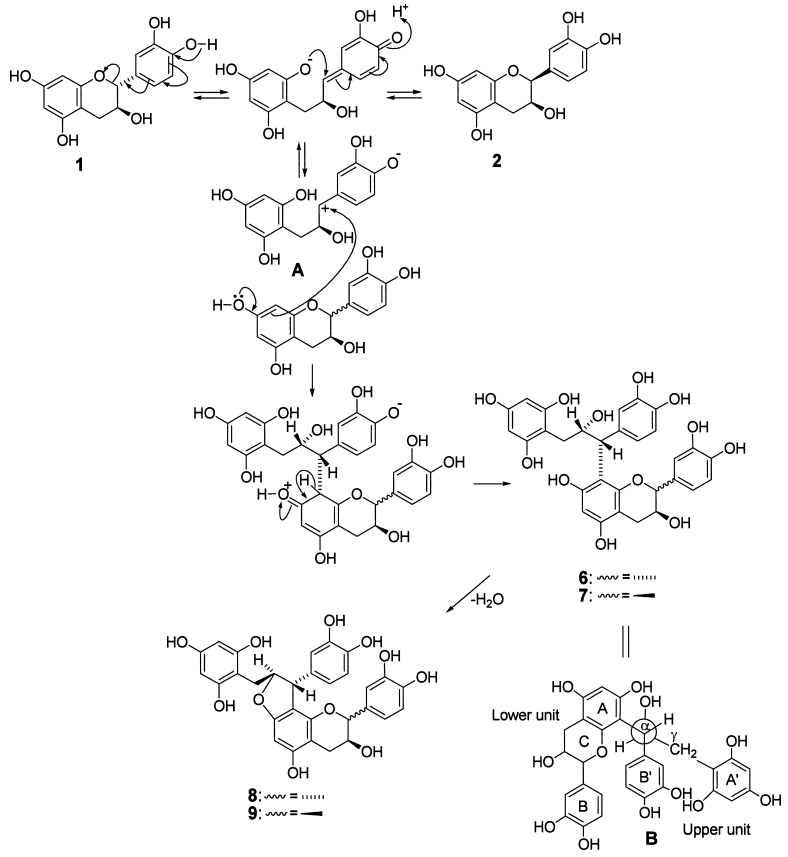

Chart 1. Proposed Mechanism for the Formation of Chalcane-Flavan Dimers from **1**

tion **B**, relative to the antipode at the α-carbon.

Weinges and coworkers[13] reported that catechin was transformed to dicatechins, which had the same plane structures as those of gambiriins A1 and A2. The dicatechins were also transformed to the corresponding anhydro-derivatives, which had the same plane structures as gambiriins B1 and B2.[13] These compounds are assumed to be identical with the compounds discussed here.

Our preliminary analyses of gambir products indicated that they also comprised oligomeric and polymeric compounds, and their presence increased following extended treatment in the manufacturing process. The structural findings obtained in this study may prove useful for investigating such higher-molecular polyphenolics.

Experimental

General Procedures Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. CD spectra were recorded on a JASCO J-720 spectrophotometer. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) spectra were recorded on a Varian INOVA AS600 spectrometer. ESI-Mass spectra were recorded on a Micromass AutoSpec OA-Tof spectrometer with the positive-ion mode (solvent, 50% MeOH+0.1% $AcONH_4$; flow rate, 20 μl/min). Column chromatography was carried out on Dia-ion HP-20 (Mitsubishi Chemical), Toyopearl HW-40 (TOSOH), MCI-gel CHP-20P (Mitsubishi Chemical), Sephadex LH-20 (Amersham Biosciences), and Chromatorex ODS (Fuji Silysia).

Extraction and Isolation A manufactured product of gambir (2 kg) purchased from Tochimoto-tenkai-do, Osaka, Japan (lot no. 171203) was homogenized in MeOH (22 l) at room temperature to give the extract (1.3 kg). A part of the extract (309 g) was subjected to a Dia-ion HP-20 column (10×60 cm; solvent, H_2O–MeOH in a stepwise-gradient mode). (+)-Catechin (**1**) was crystallized from the eluate (61 g) with 20% MeOH, and the mother liquor (14 g) was chromatographed on Toyopearl HW-40 (3.0×40 cm; solvent, 70% EtOH) to yield a fraction containing monomeric and dimeric flavans (2 g). The fraction was further chromatographed on a ODS column (solvent, 10—30% MeOH), and combined fractions (frs) 21—37 (409 mg), 38—47 (167 mg), and 51—78 (849 mg) from the column were further chromatographed on ODS and Sephadex LH-20 columns to give procyanidin B3 (**3**) (58 mg) (from frs 21—37), procyanidin B1 (**4**) (26 mg) (from frs 38—47), and gambiriin A1 (**6**) (90 mg), and gambiriin C (**5**) (17 mg) (from frs 51—78). Combined frs 79—99 (139 mg) were further chromatographed on silica gel-ODS and MCI-gel CHP-20P to give (+)-epicatechin (**2**) (12 mg). The eluate with 40% MeOH (52 g) from the Dia-ion HP-20 column was chromatographed on a Toyopearl HW-40 column (3.0×40 cm; solvent, 70% EtOH) to produce a fraction containing dimers (4.5 g). The fraction was chromatographed on MCI-gel CHP-20P column (solvent, 30—50% MeOH), and combined frs 33—103 (1.6 g) were further chromatographed on ODS, and Toyopearl HW-40 and Sephadex LH-20 columns to give gambiriin A2 (**7**) (57 mg). Combined frs 133—186 (544 mg) from the MCI-gel column were chromatographed on Toyopearl HW-40, Sephadex LH-20, and MCI-gel CHP-20P columns, and further purified by HPLC on an YMC Pack ODS A-324 column (10×300 mm) with the solvent H_2O–HCOOH–CH_3CN (81.95 : 0.05 : 18) and H_2O–HCOOH–MeOH (74.95 : 0.05 : 25) to give gambiriin B1 (**8**) (8.8 mg) and gambiriin B2 (**9**) (9.8 mg).

Products from (+)-Catechin on Heating (+)-Catechin (**1**) (1 g) in H_2O (10 ml) was autoclaved at 121 °C for 2 h. The unreacted (+)-catechin (**1**) (660 mg) was removed by crystallization and the mother liquor was chromatographed on a Toyopearl HW-40 column (2.2×40 cm; solvent, 70% EtOH). Combined frs 1—45 (232 mg) were chromatographed on MCI gel CHP-20P column, and further purified by crystallization to give (+)-epicatechin (**2**) (52 mg). Combined frs 46—105 (129 mg) were chromatographed on MCI gel CHP-20P column to give gambiriin A1 (**6**) (38 mg), and a fraction containing gambiriin A2 (32 mg) was further purified by preparative HPLC to give **7** (13 mg).

In a separate experiment, (+)-catechin (**1**) (4 g) was treated in a similar way, and a fraction containing dimers from column chromatography on Toyopearl HW-40 (2.2×40 cm; solvent, 70% EtOH) was further purified by column chromatography on MCI-gel CHP-20P, and preparative HPLC of the

fraction yielded gambiriin B1 (**8**) (3.7 mg) and gambiriin B2 (**9**) (2.5 mg).

Dehydration of 6 and 7 to Yield 8 and 9 Gambiriin A1 (**6**) (2 mg) in dry dioxane (1 ml) was heated at 50 °C in the presence of polyphosphoric acid (20 mg). The reaction mixture was subjected to preparative HPLC, to give gambiriin B1 (**8**). The identity of the compound was shown by NP and RP-HPLC and ^{1}H-NMR spectrum. Gambiriin A2 (**7**) was treated in a similar way, to give gambiriin B2 (**9**).

Gambiriin A1 (**6**): A brown amorphous powder, $[\alpha]_D$ −8.3° (c=1.0, acetone). ESI-MS m/z: 581 ($[M+H]^+$), 603 ($[M+Na]^+$), 619 ($[M+K]^+$). CD (MeOH): $[\theta]_{201}$ +1.8×10^5, $[\theta]_{213}$ −3.9×10^4, $[\theta]_{231}$ −4.2×10^4, $[\theta]_{279}$ −5.3×10^3. ^{1}H-NMR (600 MHz, acetone-d_6+D_2O, 40 °C) δ: 2.56 (1H, m, H-γa_U), 2.61 (1H, dd, J=8.5, 16.0 Hz, H-4a$_L$), 2.95 (2H, dd, J=5.0, 16.0 Hz, H-4b$_L$, H-γb_U), 3.98 (1H, q like, $J_{3,4b}$=5.0, $J_{2,3}$=7.5, $J_{3,4a}$=8.5 Hz, H-3$_L$), 4.63 (1H, br s, H-2$_L$), 4.68 (1H, m, H-β_U), 4.75 (1H, m, H-α_U), 5.95 (2H, s, H-3$_U$, H-5$_U$), 6.10 (1H, s, H-6$_L$), 6.65 (1H, d, J=8.5 Hz, H-5′$_U$), 6.68 (2H, m, H-6′$_L$, H-6′$_U$), 6.71 (1H, d, J=8.5 Hz, H-5′$_L$), 6.83 (1H, d, J=2.0 Hz, H-2′$_U$), 6.87 (1H, d, J=2.0 Hz, H-2′$_L$). ^{13}C-NMR (150 MHz, acetone-d_6+D_2O, 40 °C) δ: 29.0 (C-4$_L$), 30.1 (C-γ_U), 46.1 (C-α_U), 68.3 (C-3$_L$), 76.8 (C-β_U), 82.5 (C-2$_L$), 96.1 (2C, C-3$_U$, C-5$_U$), 97.5 (C-6$_L$), 100.5 (C-10$_L$), 105.5 (C-1$_U$), 107.2 (C-8$_L$), 115.1 (C-2′$_L$), 115.5 (C-5′$_U$), 115.7 (C-5′$_L$), 116.7 (C-2′$_U$), 119.9 (C-6′$_L$), 120.7 (C-6′$_U$), 132.1 (C-1′$_L$), 135.5 (C-1′$_U$), 143.6 (C-4′$_U$), 145.1 (C-3′$_U$), 145.4, 145.5 (C-3′$_L$, C-4′$_L$), 154.7 (C-9$_L$), 155.3 (C-5$_L$), 155.8 (C-7$_L$), 157.5 (C-4$_U$), 157.8 (2C, C-2$_U$, C-6$_U$).

Gambiriin B1 (**8**): A brown amorphous powder, $[\alpha]_D$ −39.6° (c=0.2, acetone). ESI-MS m/z: 563 ($[M+H]^+$), 585 ($[M+Na]^+$). CD (MeOH): $[\theta]_{204}$ +1.5×10^5, $[\theta]_{221}$ −4.5×10^4, $[\theta]_{245}$ −3.8×10^4, $[\theta]_{281}$ −9.7×10^3. ^{1}H-NMR (600 MHz, acetone-d_6+D_2O, 27 °C) δ: 2.49 (1H, dd, J=8.5, 15.5 Hz, H-4a$_L$), 2.76 (1H, dd, J=5.5, 15.5 Hz, H-4b$_L$), 2.88 (1H, dd, J=5.5, 12.5 Hz, H-γa_U), 2.94 (1H, dd, J=8.5, 12.5 Hz, H-γb_U), 3.77 (1H, ddd, J=5.5, 7.5, 8.4 Hz, H-3$_L$), 4.26 (1H, d, J=3.6 Hz, H-α_U), 4.55 (1H, d, J=7.5 Hz, H-2$_L$), 4.79 (1H, ddd, J=3.5, 5.5, 8.5 Hz, H-β_U), 5.92 (2H, s, H-3$_U$, H-5$_U$), 6.01 (1H, s, H-6$_L$), 6.20 (1H, dd, J=2.5, 8.5 Hz, H-6′$_U$), 6.24 (1H, dd, J=2.5, 8.5 Hz. H-6′$_L$), 6.41 (1H, d, J=2.5 Hz, H-2′$_U$), 6.56 (1H, d, J=8.5 Hz, H-5′$_L$), 6.58 (1H, d, J=8.5 Hz, H-5′$_U$), 6.64 (1H, d, J=2.5 Hz, H-2′$_L$). ^{13}C-NMR (150 MHz, acetone-d_6+D_2O, 27 °C) δ: 28.4 (C-4$_L$), 29.3 (C-γ_U), 50.0 (C-α_U), 68.4 (C-3$_L$), 81.7 (C-2$_L$), 90.4 (C-6$_L$), 92.6 (C-β_U), 95.3 (2C, C-3$_U$, C-5$_U$), 100.7 (C-10$_L$), 102.2 (C-1$_U$), 108.1 (C-8$_L$), 115.0 (C-2′$_L$), 115.2 (C-2′$_U$), 115.58 (C-5′$_L$), 115.60 (C-5′$_U$), 119.0 (C-6′$_L$), 119.6 (C-6′$_U$), 131.9 (C-1′$_L$), 137.5 (C-1′$_U$), 144.9, 145.1 (C-3′$_L$, C-4′$_L$), 143.8, 145.2 (C-3′$_U$, C-4′$_U$), 152.4 (C-9$_L$), 156.8 (C-5$_L$), 157.6 (C-4$_U$), 158.0 (2C, C-2$_U$,C-6$_U$), 159.9 (C-7$_L$).

Gambiriin A2 (**7**): A brown amorphous powder, $[\alpha]_D$ +75.7° (c=0.5, acetone). ESI-MS m/z: 581 ($[M+H]^+$), 603 ($[M+Na]^+$). CD (MeOH): $[\theta]_{204}$ +1.1×10^5, $[\theta]_{219}$ +2.3×10^{4}s, $[\theta]_{234}$ −1.5×10^4, $[\theta]_{271}$ −2.0×10^3, $[\theta]_{289}$ +3.7×10^3. ^{1}H-NMR (600 MHz, acetone-d_6+D_2O, 40 °C) δ: 2.63 (1H, m, H-γa_U), 2.81 (1H, d, J=16.0 Hz, H-4a$_L$), 2.90 (1H, dd, J=5.0, 16.0 Hz, H-4b$_L$), 3.12 (1H, dd, J=3.0, 14.5 Hz, H-γb_U), 4.20 (1H, s, H-3$_L$), 4.71 (1H, m, H-β_U), 4.81 (1H, br s, H-2$_L$), 4.89 (1H, br s, H-α_U), 5.96 (2H, br s, H-3$_U$, H-5$_U$), 6.08 (1H, s, H-6$_L$), 6.59 (1H, d, J=8.0 Hz, H-5′$_U$), 6.63 (1H, br s, H-6′$_U$), 6.76 (1H, d, J=8.0 Hz, H-5′$_L$), 6.78 (1H, br s, H-2′$_U$), 6.87 (1H, br s, H-6′$_L$), 6.99 (1H, br s, H-2′$_L$). ^{13}C-NMR (150 MHz, acetone-d_6+D_2O, 40 °C) δ: 30.4 (C-γ_U), 45.7 (C-α_U), 66.9 (C-3$_L$), 77.1 (C-β_U), 79.6 (C-2$_L$), 96.3 (2C, C-3$_U$, C-5$_U$), 97.5 (C-6$_L$), 99.4 (C-10$_L$), 105.8 (C-1$_U$), 107.2 (C-8$_L$), 114.8 (C-2′$_L$), 115.4 (C-5′$_U$), 115.7 (C-5′$_L$), 116.6 (C-2′$_U$), 119.4 (C-6′$_L$), 120.6 (C-6′$_U$), 132.2 (C-1′$_L$), 135.6 (C-1′$_U$), [143.5, 145.1 (2C), 145.3 (C-3′$_L$, C-4′$_L$, C-3′$_U$, C-4′$_U$)], 155.0 (C-9$_L$), 155.9, 156.0 (C-5$_L$, C-7$_L$), 157.5 (C-4$_U$), 157.7 (2C, C-2$_U$, C-6$_U$). The C-4$_L$ signals were overlapped with solvent peaks at δ 29—30.

Gambiriin B2 (**9**): A brown amorphous powder, $[\alpha]_D$ +136.0° (c=0.5, acetone). ESI-MS m/z: 580 ($[M+NH_4]^+$), 585 ($[M+Na]^+$). CD (MeOH): $[\theta]_{210}$ +1.8×10^5, $[\theta]_{247}$ −7.7×10^3, $[\theta]_{290}$ +4.6×10^3. ^{1}H-NMR (600 MHz, acetone-d_6+D_2O, 27 °C) δ: 2.68 (1H, dd, J=3.5, 17.0 Hz, H-4a$_L$), 2.81 (1H, dd, J=5.0, 17.0 Hz, H-4b$_L$), 2.89 (1H, dd, J=6.0, 13.0 Hz, H-γa_U), 2.98 (1H, dd, J=8.5, 13.0 Hz, H-γb_U), 4.22 (1H, dt, J=2.0, 4.0 Hz, H-3$_L$), 4.38 (1H, d, J=3.5 Hz, H-α_U), 4.71 (1H, br s, H-2$_L$), 4.77 (1H, ddd, J=3.5, 6.0, 8.5 Hz, H-β_U), 5.96 (2H, s, H-3$_U$, H-5$_U$), 6.03 (1H, s, H-6$_L$), 6.20 (1H, dd, J=2.0, 8.0 Hz, H-6′$_U$), 6.38 (1H, d, J=2.0 Hz, H-2′$_U$), 6.52 (1H, d, J=8.0 Hz, H-5′$_U$), 6.62 (1H, dd, J=2.0, 8.0 Hz, H-6′$_L$), 6.68 (1H, d, J=8.0 Hz, H-5′$_L$), 6.91 (1H, d, J=2.0 Hz, H-2′$_L$). ^{13}C-NMR (150 MHz, acetone-d_6+D_2O, 27 °C) δ: 28.9 (C-4$_L$), 29.3 (C-γ_U), 50.0 (C-α_U), 66.2 (C-3$_L$), 78.5 (C-2$_L$), 90.6 (C-6$_L$), 92.9 (C-β_U), 95.3 (2C, C-3$_U$, C-5$_U$), 100.3 (C-9$_L$), 102.4 (C-1$_U$), 108.2 (C-8$_L$), 114.8 (C-2′$_L$), 114.9 (C-2′$_U$), 115.4 (C-5′$_L$), 115.5 (C-5′$_U$), 119.1 (C-6′$_L$), 119.2 (C-6′$_U$), 131.6 (C-1′$_L$), 137.7 (C-1′$_U$), 144.8 (C-4′$_L$), 145.0 (C-3′$_L$), 143.7, 145.3 (C-3′$_U$, C-4′$_U$), 153.0 (C-9$_L$), 157.2 (C-5$_L$), 157.7 (C-4$_U$), 158.0 (2C, C-2$_U$, C-6$_U$), 160.0 (C-7$_L$).

References and Notes

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