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NEW DIMERIC FLAVANS FROM GAMBIR, AN EXTRACT OF *UNCARIA GAMBIR* †

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Abstract – Three new dimeric flavans, catechin-(4α→8)-*ent*-epicatechin (7), gambirflavan D1 (8), and gambirflavan D2 (9), were isolated from gambir (an extract from the leaves and young twigs of *Uncaria gambir*), and their structures were determined based on spectroscopic and chemical data.

INTRODUCTION

Gambir, an extract from the leaves and young twigs of *Uncaria gambir* Roxb. (Rubiaceae), is used as a natural medicine in Asian countries.[1] We previously reported the isolation of nine polyphenolic constituents from this medicine,[2] five of which were identified as procyanidins B1 and B3, gambiriin C (dimeric proanthocyanidins), (+)-catechin (**1**), and (+)-epicatechin (**2**) (monomeric compounds). Although the remaining four were considered to be the known flavan–chalcane dimers, gambiriins A1 (**3**), A2 (**4**), B1 (**5**), and B2 (**6**), our investigation revealed that their stereostructures should be revised to those shown in formulae **3**–**6** (Figure 1).[2]

Further investigation of the constituents of gambir led to the isolation of three new polyphenolic compounds, catechin-(4α→8)-*ent*-epicatechin (**7**), gambirflavan D1 (**8**), and gambirflavan D2 (**9**). This paper describes their structural determination, including the stereochemistry of the dimers, based on spectral data, syntheses, and/or chemical interconversion.

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† Dedicated to Professor Dr. Ekkehard Winterfeldt on the occasion of his 75th birthday.

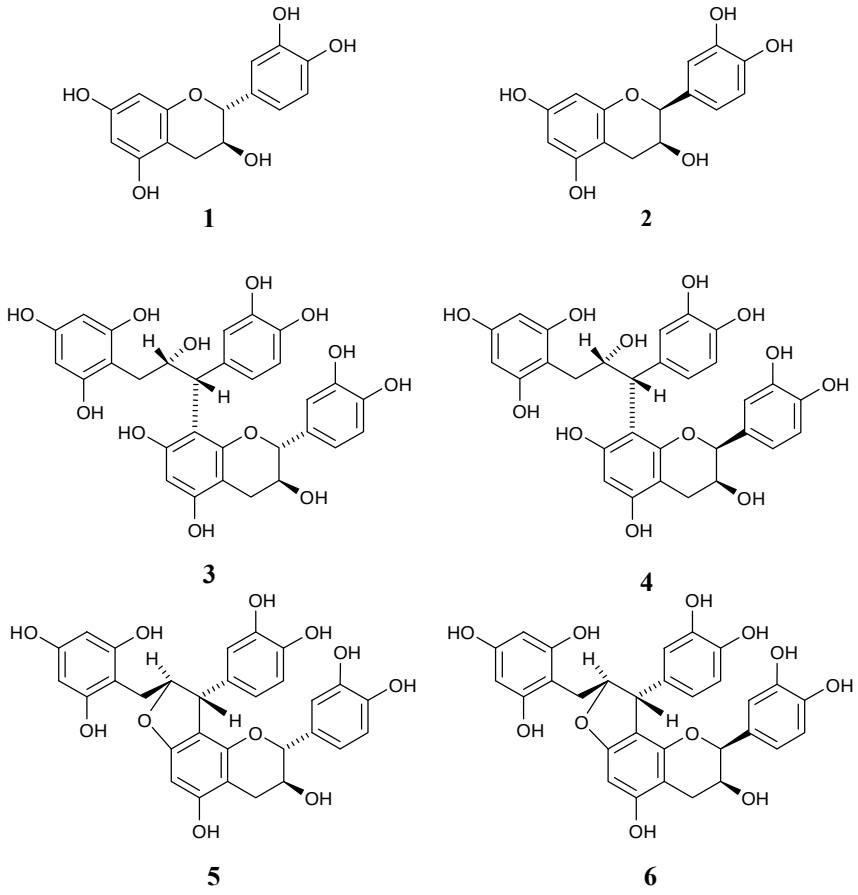

Figure 1. Structures of compounds **1**–**6** isolated from gambir (*Uncaria gambir* extract).

RESULTS AND DISCUSSION

A MeOH extract of gambir was fractionated by column chromatography on a Dia-ion HP-20, with increasing concentrations of aqueous MeOH. The eluates from the column were further purified by column chromatography with a Toyopearl HW-40, Sephadex LH-20, Chromatorex ODS-gel, and MCI-gel CHP-20P, and also by preparative HPLC to yield **7**–**9** (Figure 2), along with the compounds previously reported.[2]

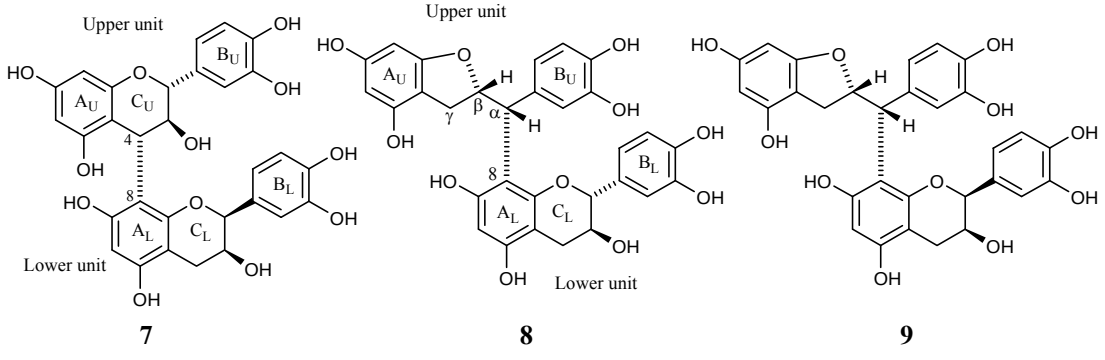

Figure 2. Structures of compounds **7**–**9** newly isolated from gambir.

Structure of 7

Catechin-(4α→8)-*ent*-epicatechin (**7**) was obtained as a brown amorphous powder. Its molecular formula, $C_{30}H_{26}O_{12}$, which corresponds to a dimeric procyanidin structure, was shown by the $[M+NH_4]^+$ ion peak in high-resolution (HR)-electrospray ionization (ESI)-MS. The ^{1}H-NMR spectrum of **7** (in acetone-d_6 containing ca. 3% D_2O) showed duplication of each signal, attributable to rotational isomerism around the sterically hindered interflavan linkage. This spectrum was quite similar to that of procyanidin B4 [catechin-(4α→8)-epicatechin].[3] The NMR spectrum of **7** was then recorded using acetone-d_6-D_2O (1:9) as the solvent to facilitate its interpretation, with spectral simplification achieved by assessing procyanidin B4 in the same solvent.[3] The ^{1}H-NMR spectrum of **7** under these conditions displayed signals of the A ring [δ 5.69, 5.45 (each, d, J = 2.0 Hz, H-6$_U$ and H-8$_U$), 6.02 (s, H-6$_L$), (U and L are the upper and lower units, respectively)], the B ring [δ 6.89 (d, J = 2.0 Hz, H-2'$_U$), 6.79 (d, J = 8.0 Hz, H-5'$_U$), 6.75 (dd, J = 2.0, 8.0 Hz, H-6'$_U$); δ 6.73 (d, J = 2.0 Hz, H-2'$_L$), 6.72 (d, J = 8.0 Hz, H-5'$_L$), 6.68 (dd, J = 2.0, 8.0 Hz, H-6'$_L$)], and the C ring [δ 4.38 (m, H-2$_U$), 4.40 (m, H-3$_U$), 4.31 (m, H-4$_U$); δ 4.28 (br s, H-2$_L$), 4.10 (m, H-3$_L$), 2.55 (dd, J = 2.5, 17.5 Hz, H-4a$_L$), 2.75 (dd, J = 5.0, 17.5 Hz, H-4b$_L$)] protons of the major conformer. Discriminating the protons of the two B rings (B_U and B_L rings) was enabled by long-range ^{1}H-^{1}H correlations, H-2$_L$/H-2'$_L$ and H-2$_L$/H-6'$_L$, in the ^{1}H-^{1}H COSY spectrum. Although the coupling patterns of the protons in the C_U ring were obscured by the partial overlapping of the H-2$_U$ and H-3$_U$ signals, measurement in acetone-d_6-D_2O (1:6) separated these protons, clearly showing their signal patterns: δ 4.38 (d, J = 10.0 Hz, H-2$_U$), 4.42 (dd, J = 8.5, 10.0 Hz, H-3$_U$), and 4.31 (d, J = 8.5 Hz, H-4$_U$). These data indicated that **7** was a procyanidin dimer composed of the 2,3-*trans* catechin (upper) and 2,3-*cis* epicatechin (lower) units. The presence of the 2,3-*trans* (upper) and 2,3-*cis* (lower) units was substantiated by the chemical shifts of the C-2$_U$ (δ 82.5) and C-2$_L$ (δ 78.3) signals in the ^{13}C-NMR spectrum.[4]

The 4→8 linkage between the upper and lower units was assigned based on the complex NMR signals resulting from the hindered rotation around the interflavan linkage, as described above. This assignment was substantiated by rotating frame nuclear Overhauser effect (ROE) interactions, H-3$_U$/H-2'$_L$ and H-3$_U$/H-6'$_L$ [Figure 3 (a)], in the ROESY spectrum, indicating spatial proximity of the B_L ring to the upper unit. The correlations H-2$_L$/C-9$_L$ and H-4$_U$/C-9$_L$ [Figure 3 (b)] in the heteronuclear multiple-bond coherence (HMBC) spectrum also substantiated the 4→8 interflavan linkage.

The 4α configuration of the interflavan linkage (configuration at C-4$_U$) in **7** was demonstrated by a negative Cotton effect in the short wavelength region ($[\theta]_{213}$ -1.1×10^5) in the CD spectrum.[5] Based on these data, **7** was determined to be catechin-(4α→8)-*ent*-epicatechin.[6]

To establish the stereochemistry of its asymmetric centers, **7** was synthesized as follows. Dihydroquercetin was treated with $NaBH_4$ and the product was heated in the presence of (+)-epicatechin (**2**) to give **7**.

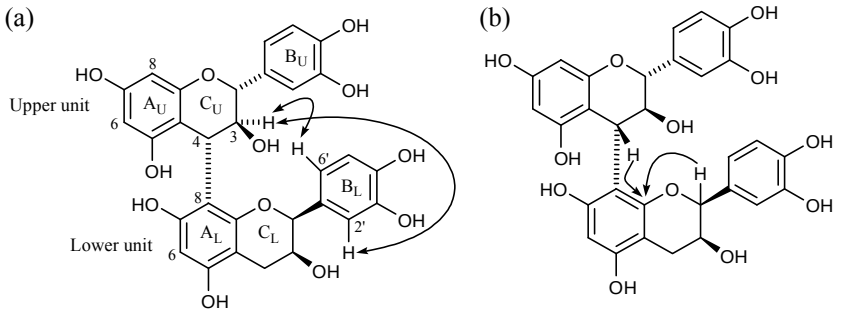

Figure 3. Important 2D-NMR correlations observed for **7**, indicating the location (C-4_U→C-8_L) of the flavan linkage, (a) ROE (H↔H) and (b) HMBC (H→C).

Structure of gambirflavan D1 (8)

Gambirflavan D1 (**8**) was obtained as an amorphous powder. The molecular formula $C_{30}H_{26}O_{11}$ was determined by HR-ESI-MS. The ^{1}H-NMR spectrum of **8** displayed two sets of ABX protons [δ 6.98 (br s, H-2'$_U$), 6.62 (d, *J* = 8.5 Hz, H-5'$_U$), 6.74 (m, H-6'$_U$); δ 6.96 (br s, H-2'$_L$), 6.76 (d, *J* = 8.0 Hz, H-5'$_L$), 6.79 (m, H-6'$_L$)], a singlet (δ 6.05, H-6_L), and two *meta*-coupled doublets [δ 5.72 (d, *J* = 2.0 Hz, H-3_U), 5.79 (d, *J* = 2.0 Hz, H-5_U)] in the aromatic region. These signals and two sets of the methine–methine–methylene protons [δ 4.58 (d, *J* = 8.5 Hz, H-α_U), 5.96 (q, *J* = 8.5 Hz, H-β_U), 2.60 (dd, *J* = 8.5, 15.0 Hz, H-γa_U), 2.86 (dd, *J* = 8.5, 15.0 Hz, H-γb_U); δ 4.50 (d, *J* = 8.0 Hz, H-2_L), 3.93 (m, H-3_L), 2.51 (dd, *J* = 9.0, 16.0 Hz, H-$4a_L$), 2.94 (dd, *J* = 6.0, 16.0 Hz, H-$4b_L$)] were observed in the spectrum. The 2,3-*trans* structure in the lower unit was indicated by a coupling constant of 8 Hz between H-2_L and H-3_L, and also by the chemical shift of the C-2_L signal (δ 82.5) in the ^{13}C-NMR spectrum. These data showed that **8** has a structure related to gambiriin A1 (**3**).[2] However, the C-β proton in the upper unit showed a noticeable downfield shift [δ 4.68 (**3**) → 5.96 (**8**)]. The ^{13}C-NMR also indicated downfield shifts of C-β_U (δ 85.6) and an A ring oxygenated carbon (δ 162; C-2_U) in **8**, relative to the corresponding signals in **3**. These data indicated that **8** possessed an ether linkage with the upper β carbon. This assignment is consistent with the molecular formula shown by the MS spectrum, which was 18 mass units less than that of **3**. A change in the signal pattern from the 2H singlet of H-3_U and H-5_U in **3** into two 1H doublets in **8** indicated the formation of the ether linkage between the hydroxyl group of the A_U ring and C-β_U to give the benzofuran structure. The correlations H-β_U/C-2_U and H-3_U/C-2_U [Fig. 4 (a)] in the HMBC spectrum substantiated the presence of this ether linkage.

The C-8 location of the interflavan linkage in this dimer was indicated by the ROE interactions H-β_U/H-2'$_L$ and H-β_U/H-6'$_L$, and by the HMBC correlations H-2_L/C-9_L and H-α_U/C-9_L [Fig. 4 (a) and (b)].

Compound **8** was isolated from the product mixture formed on heating (+)-catechin (**1**). The 2*R*,3*S* configuration in the lower unit was thus assigned based on this chemical transformation. This reaction also indicated that the upper β-carbon had the same configuration as that of the corresponding C-3 carbon in **1**. The configuration of C-β was thus determined to be *S*.

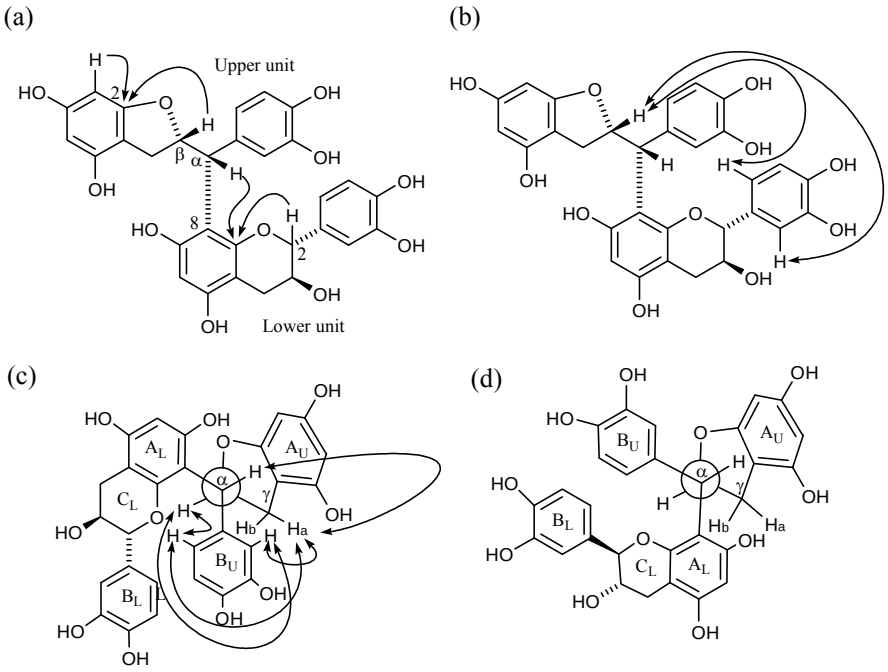

Figure 4. Important 2-D-NMR correlations observed for **8**.

(a) The HMBC correlations (H→C) indicated the presence of the ether linkage between C-2_U and C-β_U in the upper unit, and the location C-α_U→C-8_L of the interflavan linkage. (b) The ROE (H↔H) correlations indicated the C-α_U→C-8_L linkage. (c) The ROE (H↔H) correlations, H-α_U/H-γa_U, H-β_U/H-$2'_U$ (H-$6'_U$), and Hγa_U/H-$2'_U$ (H-$6'_U$) satisfied the *R* configuration at C-α_U on the assumption of the conformation based on the coupling constant between H-α_U and H-β_U (J = 9 Hz). (d) C-α_U epimer of **8**.

The remaining C-α_U configuration was assigned as follows. Considering the conformational stability around the C-α_U–C-β_U linkage, the coupling constant between H-α_U and H-β_U (J = 9 Hz) allowed two projections, as shown in Fig. 4 (c) (*R* configuration for C-α_U) and Fig. 4 (d) (*S* configuration). The ROESY spectrum showed the interactions H-α_U/H-γa_U, H-β_U/H-$2'_U$, H-β_U/H-$6'_U$, H-γa_U/H-$2'_U$, and H-γa_U/H-$6'_U$, suggesting the *R* configuration of C-α_U [Fig. 4 (c)], rather than the *S* configuration [Fig. 4 (d)]. The CD spectrum showed a positive Cotton effect ($[\theta]_{204}$ +1.1 × 10^5) in the short wavelength region. This Cotton effect corresponded to that observed for **3** ($[\theta]_{201}$ +1.8 × 10^5)[2], which has the *R* configuration at C-α_U. Formation of compound **8** upon treatment of **3** under acidic conditions was also observed. Based on these findings, structure **8** was assigned to this compound.

Structure of gambirflavan D2 (9)

Gambirflavan D2 (**9**) was obtained as an amorphous powder. The molecular formula $C_{30}H_{26}O_{11}$ shown by the $[M+NH_4]^+$ ion peak in the HR-ESI-MS was the same as that of gambirflavan D1 (**8**). The ^{1}H-NMR spectrum of **9**, which showed signals of two sets of the methine–methine–methylene protons [δ 4.77 (br s, H-α_U), 5.95 (q, *J* = 8.5 Hz, H-β_U), 2.77 (dd, *J* = 8.5, 15.0 Hz, H-γa_U), 2.99 (m, H-γb_U); δ 4.89 (br s, H-2_L), 5.74 (br s, H-3_L), 2.73 (dd, *J* = 3.0, 17.0 Hz, H-$4a_L$), 2.86 (dd, *J* = 5.0, 17.0 Hz, H-$4b_L$)], in addition to those of the phenyl groups [δ 5.74 (br s Hz, H-3_U), 5.82 (d, *J* = 2.5 Hz, H-5_U), 7.02 (br s, H-$2'_U$), 6.65 (d, *J* = 8.5 Hz, H-$5'_U$), 6.78 (br d, *J* = 8.5 Hz, H-$6'_U$), 6.02 (s, H-6_L), 7.04 (d, *J* = 2.0 Hz, H-$2'_L$), 6.76 (d, *J* = 8.5 Hz, H-$5'_L$), 6.82 (dd, *J* = 2.5, 8.5 Hz, H-$6'_L$)], was quite similar to that of **8**. However, the signals of the upper unit (H-α_U, H-γ_U, H-3_U, H-$2'_U$, H-$6'_U$) in the ^{1}H-NMR spectrum were very broad, even on measurement at 40ºC, indicating increased steric hindrance around the interflavan linkage relative to that in **8**. A difference between the ^{1}H-NMR spectra of **8** and **9** was also observed in the coupling constant between H-2_L and H-3_L [**9**: δ 4.89 (1H, br s, H-2_L) and 4.19 (1H, m, H-3_L)]. The small coupling constant (<2 Hz) and the chemical shift of the C-2_L signals (δ 79.6) in the ^{13}C-NMR spectrum indicated that the lower unit in **9** was epicatechin.

The downfield shift of the C-β proton in the upper unit [δ 5.95 (1H, q, *J* = 8.5 Hz)] in **9** indicated the position of the ether bond between the hydroxyl group at C-β_U and the hydroxyl group at C-2_U, as in **8**. The presence of the ether linkage was confirmed by the HMBC correlations H-β_U/C-2_U and H-3_U/C-2_U [Figure 5 (a)]. The C-8_L position of the interflavan linkage was shown by the interactions H-β_U/H-$2'_L$ and H-β_U/H-$6'_L$ [Figure 5 (b)] in the ROESY spectrum.

This compound also obtained by heating (+)-catechin (**1**). The 3*S* configuration in the lower unit and the *S* configuration in the upper β carbon were thus established, based on the 3*S* configuration of **1**. Because the lower unit has the 2,3-*cis* structure described above, the configuration at C-2_L should be *S*. The remaining C-α_U configuration was assigned to be *R*, in a manner similar to that in **8**. The coupling constant between H-α_U and H-β_U (*J* = 9 Hz) in **9** is consistent with the *R* configuration for C-α_U, as shown in the projection of Figure 5 (c), where the ROE interactions H-α_U/H-γa_U, H-β_U/H-$2'_U$, H-β_U/H-$6'_U$, and H-γa_U/H-$6'_U$ [Figure 5 (c)] support this configuration. Furthermore, the formation of **9** was also observed on treatment of gambiriin A2 (**4**), having the *R* configuration of C-α_U, with polyphosphoric acid. The CD spectrum of **9** showed a positive Cotton effect ($[\theta]_{206}$ $+1.4 \times 10^5$) in the short wavelength region, as in the case of **4**,[2] substantiating the *R* configuration.

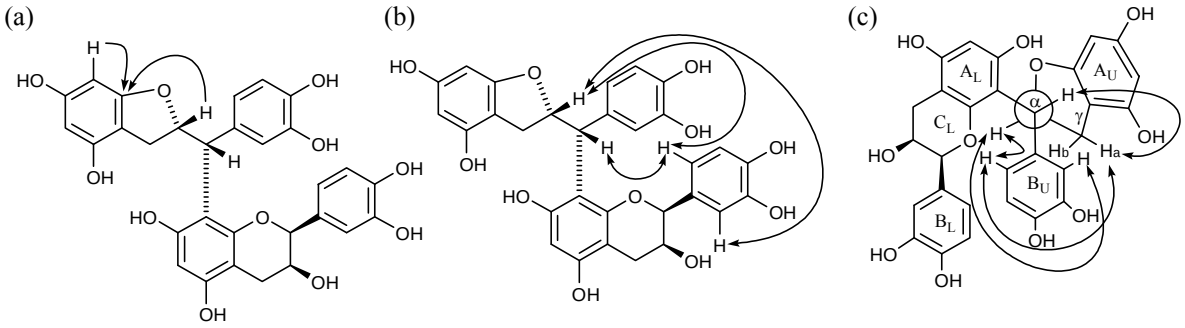

Figure 5. Important 2D-NMR correlations observed for **9**.

(a) The HMBC correlations (H→C) indicated the presence of the ether linkage between C-2_U and C-β_U in the upper unit. (b) The ROE (H↔H) correlations indicated the C-α_U→C-8_L linkage. (c) The ROE (H↔H) correlations, H-α_U/H-γa_U, H-β_U/H-$2'_U$ (H-$6'_U$), and Hγa_U/H-$2'_U$, satisfied the *R* configuration at C-α_U based on the coupling constant between H-α_U and H-β_U (J = 9 Hz).

Conclusions

In the present study, we obtained three dimeric compounds having structures related to catechin, in addition to several dimers reported in a previous paper.[2] Because gambir has been produced from leaves and young twigs by extracting with water under heating, at least in part, some of those dimers may be derived from catechin (**1**), the main constituent of gambir, or related compounds. Compound **7** may be formed by epimerization of C-2_L in procyanidin B3 [catechin-(4α→8)-catechin], which has also been isolated from gambir.[2] Compounds **8** and **9** have an ether linkage between the upper A ring and β carbon, which was structurally related to **3** and **4**. Compounds **8** and **9** were also isolated from the product mixture obtained on heating the solution of **1**. This report provides fundamental findings on the reactivity of **1** and related compounds.

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 (150 MHz) NMR spectra were recorded on a Varian INOVA AS600 spectrometer. ESI-mass spectra were taken on Micromass AutoSpec OA-Tof in positive-ion mode (solvent, 50% MeOH + 0.1% $AcONH_4$; flow rate, 20 μL/min). Column chromatography (CC) was carried out on a Dia-ion HP-20 (Mitsubishi Chemical), Toyopearl HW-40 (Tosoh), MCI-gel CHP-20P (Mitsubishi Chemical), Sephadex LH-20 (Amersham Biosciences), and Silica gel-ODS (Chromatorex).

Extraction and isolation Gambir (2 kg), purchased from Tochimoto-tenkai-do (Osaka, Japan), was

extracted with MeOH, and the extract was subjected to Dia-ion HP-20 CC.[2] The 20% MeOH eluate (14.07 g) from the Dia-ion HP-20 column was chromatographed on a column of Toyopearl HW-40, after removal of **1** by crystallization. The fraction thus obtained was further purified on silica gel-ODS, Toyopearl HW-40, and MCI-gel CHP-20P columns, and by preparative HPLC, to give catechin-(4α→8)-*ent*-epicatechin (**7**) (4.5 mg). The eluate with 40% MeOH (51.89 g) from the Dia-ion HP-20 column was chromatographed on Toyopearl HW-40, and MCI gel CHP-20P columns. Two fractions thus obtained were purified by CC on silica gel-ODS, Sephadex LH-20, Toyopearl HW-40, and/or MCI-gel CHP-20P, and also by preparative HPLC, to give gambirflavan D1 (**8**) (11.1 mg) and gambirflavan D2 (**9**) (5.8 mg).

Heating an aqueous solution of (+)-catechin (1) Compound **1** (1 g) in H_2O (10 mL) was autoclaved (121ºC, 2 h), as reported previously.[2] The mother liquor after crystallization of unreacted **1** was chromatographed on a Toyopearl HW-40 column. A fraction containing dimers was chromatographed on an MCI gel CHP-20P column and further purified by preparative HPLC to give gambirflavan D1 (**8**) (1.8 mg). Gambirflavan D2 (**9**) (1.5 mg) was obtained from 4 g of **1** in a similar way.

Synthesis of catechin-(4α→8)-*ent*-epicatechin (7) Dihydroquercetin (50 mg in 7.5 mL EtOH), which was isolated from Douglas-fir bark,[3] was treated with $NaBH_4$ (40 mg in 2.5 mL EtOH) for 30 min at room temperature under N_2 in the presence of (+)-epicatechin (**2**) (100 mg) at pH 6–7, which was adjusted with 1.5 M AcOH. The solution was diluted with 10 mL H_2O and then acidified to pH 5 with 1.5 M AcOH, and the solution was heated for 4 h at 45ºC under N_2. The solution was extracted with AcOEt and the main product was purified by Toyopearl HW-40 CC. The identity of the product (10.4 mg) with **7** was confirmed by comparing the HPLC and ^{1}H-NMR results.

Conversion of gambiriin A1 (3) into gambirflavan D1 (8), and gambiriin A2 (4) into gambirflavan D2 (9) Compound **3** (2.0 mg) in dry dioxane (1 mL) was heated at 50°C in the presence of polyphosphoric acid (20 mg) for 2 days. Formation of **8** during the reaction was shown by normal phase (NP) HPLC [t_R 6.1 min: column, YMC-Pack SIL A-003 (4.6 mm I.D. × 250 mm, YMC); solvent, *n*-hexane-THF-MeOH-HCOOH (55:33:11:1), containing oxalic acid 450 mg/L; detection, 280 nm; flow rate, 1.5 mL/min)] and reverse-phase (RP) HPLC [t_R 5.7 min: column, YMC Pack ODS A-302 (4.6 mm I.D. × 150 mm, YMC); solvent, 0.01M H_3PO_4-0.01M KH_2PO_4-CH_3CN (17:3); detection, 280 nm; flow rate, 1.0 mL/min; temperature, 40ºC]. Compound **4** was treated in a similar way, showing the formation of **9** on NP (t_R 6.1 min) and RP (t_R 7.0 min) HPLC.

Catechin-(4α→8)-*ent*-epicatechin (7) An amorphous brown powder, $[\alpha]_D$ –80.9º (*c* 0.5, acetone). ESI-MS *m/z*: 579 ($[M+H]^+$), 596 ($[M+NH_4]^+$). HR-ESI-MS *m/z*: 598.1774 ($[M+NH_4]^+$) (calculated for $C_{30}H_{26}O_{12}$+NH_4, 596.1768). CD (MeOH): $[\theta]_{213}$ –1.1 × 10^5, $[\theta]_{232}$ –3.0 × 10^4s, $[\theta]_{268}$ +1.9 × 10^3, $[\theta]_{285}$ –2.7 × 10^3. ^{1}H-NMR (600 MHz, acetone-d_6+D_2O, 27ºC) δ: [2.71 (dd, *J* = 3.0, 17.0 Hz, H-4a$_L$), 2.81 (dd, *J* = 4.5, 17.0 Hz, H-4b$_L$), 2.85 (m, H-4$_L$), 2H in total], [4.16 (m), 4.21 (m), H-3$_L$, 1H in total], [4.36 (d, *J* = 8.5 Hz), 4.41 (d, *J* = 8.5 Hz), H-2$_U$, 1H in total], [4.49 (d, *J* = 8.5 Hz), 4.64 (d, *J* = 8.5 Hz), H-4$_U$, 1H in total], [4.51 (1H, t, *J* = 8.5 Hz), 4.66 (t, *J* = 8.5Hz), H-3$_U$, 1H in total], [4.54 (br s), 4.96 (br s), H-2$_L$, 1H in total], [5.70 (d, *J* = 2.5 Hz), 5.76 (d, *J* = 2.5 Hz), 5.86 (d, *J* = 2.5 Hz), 5.87 (d, *J* = 2.5 Hz), H-6$_U$, H-8$_U$, 2H in total], [6.04 (s), 6.19 (s), H-6$_L$, 1H in total], [6.73 (d, *J* = 8.0 Hz), 6.76 (d, *J* = 8.0 Hz), H-5'$_U$, 1H in total], [6.78 (d, *J* = 8.5 Hz), 6.79 (d, *J* = 8.5 Hz), H-5'$_L$, 1H in total], [6.79 (dd, *J* = 2.0, 8.0 Hz,), 6.77 (dd, *J* = 2.0, 8.0 Hz), H-6'$_U$, 1H in total], [6.85 (dd, *J* = 2.0, 8.5 Hz), 6.91 (1H, dd, *J* = 2.0, 8.5 Hz), H-6'$_L$, 1H in total], [6.90 (1H, d, *J* = 2.0 Hz), 7.12 (d, *J* = 2.0 Hz), H-2'$_L$, 1H in total], [6.93 (d, *J* = 2.0 Hz), 7.00 (d, *J*=2.0 Hz), H-2'$_U$, 1H in total]. ^{13}C-NMR (150 MHz, acetone-d_6+D_2O, 27ºC) δ: 37.8, 38.5 (C-4$_U$), 66.0, 66.1 (C-3$_L$), 72.2, 72.6 (C-3$_U$), 79.2, 79.9 (C-2$_L$), 83.7, 83.8 (C-2$_U$), 96.2, 97.3 (C-6$_L$), 96.1, 96.2, 96.8, 97.3 (C-6$_U$, C-8$_U$, 2C in total), 99.4, 101.1 (C-10$_L$), 105.8, 106.1 (C-10$_U$), 106.6, 107.1 (C-8$_L$), 114.5, 115.2 (C-2'$_L$), 115.3, 115.4, 115.5, 115.6 (C-5'$_U$, C-5'$_L$), 115.8, 116.2 (C-2'$_U$), 119.1, 119.8 (C-6'$_L$), 120.4, 120.6 (C-6'$_U$), 131.5, 132.0 (C-1'$_L$), 132.1, 132.2 (C-1'$_U$), 144.9, 145.0, 145.1, 145.3, 145.4, 145.5, 145.6 (C-3'$_U$, C-4'$_U$, C-3'$_L$, C-4'$_L$, 4C in total), 154.7, 155.3, 155.4, 155.9, 156.1, 156.7, 156.9, 157.0, 157.3, 158.1, and 158.4 (C-5$_U$, C-7$_U$, C-9$_U$, C-5$_L$, C-7$_L$, C-9$_L$, 6C in total). The C-4$_L$ of the signals of the two rotamers overlapped with solvent peaks at δ 29-30. ^{1}H-NMR [600 MHz, acetone-d_6-D_2O (1:9), 27°C]: δ 2.55 (1H, dd, *J* = 2.5, 17.5 Hz, H-4a$_L$), 2.75 (1H, dd, *J* = 5.0, 17.5 Hz, H-4b$_L$), 4.10 (1H, m, H-3$_L$), 4.28 (1H, br s, H-2$_L$), 4.31 (1H, m, H-4$_U$), 4.38 (m, H-3$_U$), 4.40 (m, H-2$_U$), [5.45, 5.69 (each 1H, d, *J* = 2.0 Hz, H-6$_U$, H-8$_U$)], 6.02 (1H, s, H-6$_L$), 6.69 (1H, dd, *J* = 2.0, 8.0 Hz, H-6'$_L$), 6.72 (1H, d, *J* = 8.0 Hz, H-5'$_L$), 6.73 (1H, d, *J* = 2.0 Hz, H-2'$_L$), 6.75 (1H, dd, *J* = 2.0, 8.0 Hz, H-6'$_U$), 6.79 (1H, d, *J* = 8.0 Hz, H-5'$_U$), 6.89 (1H, d, *J* = 2.0 Hz, H-2'$_U$). ^{13}C-NMR [150 MHz, acetone-d_6:D_2O (1:9), 27ºC]:δ 28.1 (C-4$_L$), 37.7 (C-4$_U$), 65.6 (C-3$_L$), 72.4 (C-3$_U$), 78.3 (C-2$_L$), 82.5 (C-2$_U$), 95.5, 96.7 (C-6$_U$, C-8$_U$), 95.9 (C-6$_L$), 100.7 (C-10$_L$), 107.1 (C-10$_U$), 109.2 (C-8$_L$), 114.9 (C-2'$_L$), 115.7 (C-5'$_L$), 116.2 (C-2'$_U$), 116.5 (C-5'$_U$), 119.9 (C-6'$_L$), 121.1 (C-6'$_U$), 130.7 (C-1'$_U$), 131.3 (C-1'$_L$), 143.7, 144.3, 144.8 (C-3'$_L$, C-4'$_L$, C-3'$_U$, C-4'$_U$, 4C in total), 153.6 (C-5$_L$), 154.0 (C-7$_L$), 154.2 (C-9$_L$), 154.7 (C-7$_U$), 155.2 (C-5$_U$), and 157.1 (C-9$_U$).

Gambirflavan D1 (8) A brown amorphous powder, $[\alpha]_D$ +20.8º (*c* 0.2, acetone). ESI-MS *m/z*: 563 ($[M+H]^+$), 585 ($[M+Na]^+$). HR-ESI-MS *m/z*: 563.1551 ($[M+H]^+$) (calculated for $C_{30}H_{28}O_{12}$ + H, 563.1553). CD (MeOH): $[\theta]_{204}$ +1.1 × 10^5, $[\theta]_{234}$ –2.6 × 10^4, $[\theta]_{281}$ –6.5 × 10^3. ^{1}H-NMR (600 MHz, acetone-d_6+D_2O,

27ºC) δ: 2.51 (1H, dd, J = 9.0, 16.0 Hz, H-4a$_L$), 2.60 (1H, dd, J = 8.5, 15.0 Hz, H-γa$_U$), 2.86 (1H, dd, J = 8.5, 15.0 Hz, H-γb$_U$), 2.94 (1H, dd, J = 6.0, 16.0 Hz, H-4b$_L$), 3.93 (1H, m, H-3$_L$), 4.50 (1H, d, J = 8.0 Hz, H-2$_L$), 4.58 (1H, d, J = 8.5 Hz, H-α$_U$), 5.72 (1H, d, J = 2.0 Hz, H-3$_U$), 5.79 (1H, d, J = 2.0 Hz, H-5$_U$), 5.96 (1H, q, J = 8.5 Hz, H-β$_U$), 6.05 (1H, s, H-6$_L$), 6.62 (1H, d, J = 8.5 Hz, H-5'$_U$), 6.74 (1H, m, H-6'$_U$), 6.76 (1H, d, J = 8.0 Hz, H-5'$_L$), 6.79 (1H, m, H-6'$_L$), 6.96 (1H, br s, H-2'$_L$), and 6.98 (1H, br s, H-2'$_U$). ^{13}C-NMR (150 MHz, acetone-d_6+D_2O, 27ºC) δ: 32.8 (C-γ$_U$), 46.8 (C-α$_U$), 68.3 (C-3$_L$), 82.8 (C-2$_L$), 85.6 (C-β$_U$), 90.3 (C-3$_U$), 95.7 (C-5$_U$), 96.7 (C-6$_L$), 100.9 (C-10$_L$), 104.4, (C-1$_U$), 108.8 (C-8$_L$), 115.3 (C-5'$_U$), 115.4 (C-2'$_L$), 115.7 (C-5'$_L$), 117.2 (C-2'$_U$), 120.0 (C-6'$_L$), 121.2 (C-6'$_U$), 132.0 (C-1'$_L$), 135.3 (C-1'$_U$), 143.9, 145.2, 145.5, 145.6 (C-3'$_L$, C-4'$_L$, C-3'$_U$, C-4'$_U$), 154.76 (C-9$_L$), 154.8(C-5$_L$), 154.9 (C-7$_L$), 155.1 (C-6$_U$), 159.4 (C-4$_U$), and 162.7 (C-2$_U$). The C-4$_L$ signal overlapped with the solvent peaks at δ 29–30.

Gambirflavan D2 (9) A brown amorphous powder, $[\alpha]_D$ +70.7º (c 0.5, acetone). ESI-MS m/z: 580 ($[M+NH_4]^+$), 585 ($[M+Na]^+$). HR-ESI-MS m/z: 580.1823 ($[M+NH_4]^+$) (calculated for $C_{30}H_{26}O_{11}$ + NH_4, 580.1819). CD (MeOH): $[\theta]_{206}$ +1.4 × 10^5, $[\theta]_{222}$ +1.6 × 10^4, $[\theta]_{243}$ +1.0 × 10^4, $[\theta]_{276}$ –4.4 × 10^3. ^{1}H-NMR (600 MHz, acetone-d_6+D_2O, 40ºC) δ: 2.73 (1H, dd, J= 3.0, 17.0 Hz, H-4a$_L$), 2.77 (1H, dd, J= 8.5, 15.0 Hz, H-γa$_U$), 2.86 (1H, dd, J= 5.0, 17.0 Hz, H-4b$_L$), 2.99 (1H, m, H-γb$_U$), 4.19 (1H, m, H-3$_L$), 4.77 (1H, br s, H-α$_U$), 4.89 (1H, br s, H-2$_L$), 5.74 (1H, br s, H-3$_U$), 5.82 (1H, d, J= 2.5 Hz, H-5$_U$), 5.95 (1H, q, J= 8.5 Hz, H-β$_U$), 6.02 (1H, s, H-6$_L$), 6.65 (1H, d, J = 8.5 Hz, H-5'$_U$), 6.76 (1H, d, J= 8.5 Hz, H-5'$_L$), 6.78 (1H, br d, J= 8.5 Hz, H-6'$_U$), 6.82 (1H, dd, J= 2.0, 8.5 Hz, H-6'$_L$), 7.02 (1H, brs, H-2'$_U$), and 7.04 (1H, d, J= 2.0 Hz, H-2'$_L$). ^{13}C-NMR (150 MHz, acetone-d_6+D_2O, 40ºC) δ: 32.6 (C-γ$_U$), 46.1 (C-α$_U$), 66.7 (C-3$_L$), 79.6 (C-2$_L$), 85.8 (C-β$_U$), 90.5 (C-3$_U$), 96.0 (C-5$_U$), 97.1 (C-6$_L$), 99.9 (C-10$_L$), 104.6 (C-1$_U$), 108.3 (C-8$_L$), 115.0 (C-2'$_L$), 115.5 (C-5'$_U$), 115.7 (C-5'$_L$), 117.0 (C-2'$_U$), 119.2 (C-6'$_L$), 121.1 (C-6'$_U$), 132.0 (C-1'$_L$), 135.0 (C-1'$_U$), 143.8, 145.1, 145.2, 145.3 (C-3'$_L$, C-4'$_L$, C-3', C-$_U$4'), 154.8 (C-6$_U$), 154.9 (C-9$_L$), 155.2 (C-7$_L$), 155.5 (C-5$_L$), 159.4 (C-4$_U$), and 162.3 (C-2$_U$). The C-4$_L$ signal overlapped with the solvent peaks at δ 29–30.

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REFERENCES AND NOTES

1. L. M. Perry, Medicinal Plants of East and Southeast Asia, MIT Press, Cambridge, MA, 1980, p. 359.
2. S. Taniguchi, K. Kuroda, K. Doi, M. Tanabe, T. Shibata, T. Yoshida, and T. Hatano, *Chem. Pharm. Bull.,* 2007, **55**, 268.
3. T. Hatano and R. W. Hemingway, *J. Chem. Soc., Perkin Trans. 2,* 1997, 1035.
4. T. L. Eberhardt and R. A. Young, *J. Agric. Food. Chem*., 1994, **42**, 1704.
5. M. A. Barret, W. Klyne, P. M. Scopes, A. C. Fletcher, L. J. Poter, and E. Hsalam, *J. Chem. Soc., Perkin Trans. 1,* 1979, 2375.
6. The name for structure **7**, catechin-(4α→8)-*ent*-epicatechin, was given previously to procyanidin B4 in another paper, when the CA registry number 174589-51-6 was assigned. B. Bartolome, T., Hernandez, M. L. Bengoechea, C. Quesada, C. Gomez-Cordoves, and I. Estrella, *J. Chromatogr A,* 1996, **723**, 19. However, this was a typographical error as procyanidin B4 is catechin-(4α→8)-epicatechin.