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 gambirin 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; gambirin

Results and Discussion

A manufactured product of gambir purchased in Japan was 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.”

Gambir, an official pharmacopoeic medicine in Japan, is the aqueous extract of the leaves and young twigs of Uncaria gambir Roxb. (Rubiaceae).1) 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 compounds5) 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 1H- and 13C-NMR spectral assignments are also shown here, since those were not reported in detail previously.5,8)

The dimeric molecular formula, C30H28O12, was indicated by the [M+H]+ ion peak at m/z 581 in the electrospray-ionization (ESI)-MS. The 1H-NMR spectrum showed signals due to A- and A’-ring protons [δ: 5.95 (2H, s, H-3’L, H-5’L), 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 1H-3H correlations, H-αL/H-2’U and H-αH/H-6’U. The spectrum also showed two sets of the methine–methine–methylene connectivities in the aliphatic region. H-2L and H-3L in one set [δ: 4.63 (H-2L), 3.98 (H-3L), 2.61 (H-4aL), 2.95 (H-4bL)] 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 13C-NMR spectrum, which showed the C-2L signal at δ 82.5. The remaining set of the methine–methine–methylene protons [δ: 2.56 (H-γαU), 2.95 (H-γβU), 4.68 (H-βU), 4.75 (H-αU)] 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 rotation frame nuclear Overhauser effect spectroscopy (ROESY) of 6 showed correlations of H-αL/H-2’U and H-βL/H-2’L, indicating the presence of an interferonoid link between position C-8L and the upper chalcane unit (C-αL) (Fig. 2). These correlations would not be observed if the linkage occurred at C-6L. The aliphatic protons of 6 appeared as broad signals, due to the restricted rotation around the interferonoid linkage, which confirmed the location of the linkage at C-8L.

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 2R,3S-configurations in the lower unit and the S-configuration of the upper β carbon (C-βL) were thus confirmed. Although the 1H- and 13C-NMR spectral data suggested the identity of 6 as gambiriin A1 (6a) in the literature,3) the stereochemistry of C-αL was to be revised based on the relationship with 8, as discussed later.

Gambirin B1 (8) was obtained as a light-brown powder.
The molecular formula was determined to be C\textsubscript{30}H\textsubscript{26}O\textsubscript{11} based on the [M+H]\textsuperscript{+} ion peak at m/z 563 in the ESI-MS, with the nominal molecular weight 18 mass unit (H\textsubscript{2}O) less than that of gambirin A1 (6). The \textsuperscript{1}H-NMR spectrum showed protons of the A- and A'-rings [\(\delta\): 5.92 (2H, s, H-3\textsubscript{U}, H-5\textsubscript{U}), 6.01 (1H, s, H-6\textsubscript{L})] and two sets of ABX signals of the B- and B'-ring protons [\(\delta\): 6.20 (1H, dd, \(J=2.5, 8.5\) Hz, H-6'\textsubscript{U}), 6.24 (1H, dd, \(J=2.5, 8.5\) Hz, H-6'\textsubscript{L}), 6.41 (1H, d, \(J=2.5\) Hz, H-2'\textsubscript{U}), 6.56 (1H, d, \(J=8.5\) Hz, H-5'\textsubscript{U}), 6.58 (1H, d, \(J=8.5\) Hz, H-5'\textsubscript{L}), 6.64 (1H, d, \(J=2.5\) Hz, H-2'\textsubscript{L})]. The spectrum also showed two sets of the methine–methine–methylene systems, which were assigned to be a C-ring [\(\delta\): 4.55 (1H, d, \(J=7.5\) Hz, H-2\textsubscript{L}), 3.77 (1H, ddd, \(J=5.5, 7.5, 8.5\) Hz, H-3\textsubscript{L}), 2.49 (1H, dd, \(J=8.5, 15.5\) Hz, H-4\textsubscript{aL}), 2.76 (1H, dd, \(J=5.5, 15.5\) Hz, H-4\textsubscript{bL})] of the catechin residue and a substituted propyl group [\(\delta\): 4.26 (1H, d, \(J=3.5\) Hz, H-\(\alpha\textsubscript{U}\)), 4.79 (1H, ddd, \(J=3.5, 5.5, 8.5\) Hz, H-\(\beta\textsubscript{U}\)), 2.88 (1H, dd, \(J=5.5, 12.5\) Hz, H-\(\gamma\textsubscript{aU}\)), 2.94 (1H, dd, \(J=8.5, 12.5\) Hz, H-\(\gamma\textsubscript{bU}\))] of the chalcone structure.

The \textsuperscript{13}C-NMR data also showed that 8 was composed of the chalcone 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-\(\alpha\textsubscript{U}\)/C-7\textsubscript{L}, H-\(\alpha\textsubscript{U}\)/C-9\textsubscript{L}, H-\(\beta\textsubscript{U}\)/C-7\textsubscript{L}, H-2\textsubscript{L}/C-9\textsubscript{L}, H-4\textsubscript{L}/C-5\textsubscript{L}, H-4\textsubscript{L}/C-9\textsubscript{L}, H-6\textsubscript{L}/C-5\textsubscript{L}, and H-6\textsubscript{L}/C-7\textsubscript{L}. The location of the interflavonoid linkage between C-\(\alpha\textsubscript{U}\) and C-8\textsubscript{L} was indicated by the correlations H-\(\alpha\textsubscript{U}\)/C-9\textsubscript{L} and H-2\textsubscript{L}/C-9\textsubscript{L}.

The molecular formula shown by the ESI-MS and the downfield shift of an A-ring carbon (\(\delta\) 159.9; C-7\textsubscript{L}) of 8, relative to the corresponding carbon of 6, suggested that 8 possessed an ether linkage at C-7\textsubscript{L}. The HMBC correlation H-\(\beta\textsubscript{U}\)/C-7\textsubscript{L} indicated that the ether bond formed between the hydroxyl group at C-\(\beta\textsubscript{U}\) and the hydroxyl group at C-7\textsubscript{L}. The \textsuperscript{1}H-NMR spectrum showed sharp signals for the aliphatic protons in spite of the location of the interflavonoid linkage at C-8\textsubscript{L}, and the fixed conformation due to the presence of the ether linkage enabled the rationalization of this phenome-
The molecular formula was determined to be C$_{30}$H$_{26}$O$_{11}$.

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+Na]$^+$ and 603 [M+Na]$^+$. The 1H-NMR spectrum of 7, which was similar to that of 6, indicated that this compound was also a chalcone-flavan dimer. The differences in the 1H-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$_U$), 4.20 (1H, br s, H-3$_U$), 2.81 (1H, d, J=16.0 Hz, H-4$_U$), 2.90 (1H, dd, J=5.0, 16.0 Hz, H-4b$_U$)] in the aliphatic region of 7. The small coupling constant between H-2$_U$ and H-3$_U$ indicated the 2,3-cis configuration of the lower unit. The 13C-NMR spectrum also showed the signals of the chalcone and flavan structure and the chemical shift of C-2$_U$ (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$_U$, 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'$_U$, H-α$_U$/H-6'$_U$, H-β$_U$/H-2'$_U$, and H-β$_U$/H-6'$_U$).

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, and the lower unit as having the 2S,3S-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$_{28}$O$_{11}$ based on the ion peak at m/z 580 [M+Na]$^+$ and 585 [M+Na]$^+$ in the ESI-MS. The nominal molecular weight 562 was the same as that of gambiriin B1 (8). Although the 1H- and 13C-NMR spectra of 9 were similar to those of gambiriin B1 (8), the differences in the 1H- and 13C-NMR spectra between 8 and 9 were among the 1H and 13C signals of the lower unit C-ring. The 1H signals of the lower unit, which appeared at δ 4.71 (br s, H-2$_U$), 4.22 (m, H-3$_U$), 2.68 (dd, J=3.5, 17.0 Hz, H-4$_U$), 2.81 (dd, J=5.0, 17.0 Hz, H-4b$_U$), indicated that the lower unit was 2,3-cis epicatechin in 9 instead of 2,3-trans catechin in 8. The 13C chemical shift of C-2$_U$ (δ 78.5) also satisfied the 2,3-cis structure. The HMBC correlations, H-2$_U$/C-9$_U$ and H-α$_U$/C-9$_U$, showed that the position of the interflavonoid linkage on the lower unit was at C-8$_U$. The formation of the ether bond between the hydroxyl group at C-β$_U$ and the hydroxyl group at C-7$_U$ was demonstrated by the correlation H-β$_U$/C-7$_U$ in the HMBC spectrum.

Compound 9 was also obtained from (±)-catechin (1) on the heating reaction. The 2S,3S-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. 31 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 from 1 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-
tion B, relative to the antipode at the α-carbon.

Weinges and coworkers\textsuperscript{13) reported that catechin was transformed to dicatechins, which had the same plane structures as those of gambirins A1 and A2. The dicatechins were also transformed to the corresponding anhydro-derivatives, which had the same plane structures as gambirins B1 and B2.\textsuperscript{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. \textsuperscript{1}H- (600 MHz) and \textsuperscript{13}C-NMR (150 MHz) spectra were recorded on a Varian INOA AS600 spectrometer. ESI-Mass spectra were recorded on a Micromass AutoSpec OA-Tof spectrometer with the positive-ion mode (solvent, 50% MeOH/H\textsubscript{2}O 0.1% AcONH\textsubscript{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\textsubscript{2}O–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 procyandin B3 (3) (58 mg) (from frs 21—37), procyandin B1 (4) (26 mg) (from frs 38—47), and gambirin A1 (6) (90 mg), and gambirin 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 gambirin 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\textsubscript{2}O–HCOOH–MeOH (74.95 : 0.05 : 25) to give gambirin B1 (8) (8.8 mg) and gambirin B2 (9) (9.8 mg).

**Products from (+)-Catechin on Heating** (+)-Catechin (1) (1 g) in H\textsubscript{2}O (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 gambirin A1 (6) (38 mg), and a fraction containing gambirin 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 gambirin B1 (8) (3.7 mg) and gambirin B2 (9) (2.5 mg).

Dehydration of 6 and 7 to Yield 8 and 9 Gambirin 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 gambirin B1 (8). The identity of the compound was shown by NP and RP-HPLC and 1H-NMR spectrum. Gambirin A2 (7) was treated in a similar way, to give gambirin B2 (9).

Gambirin A1 (6): A brown amorphous powder, \( \text{IR} \) (KBr): 3043—3406 (1968). ESI-MS m/z: 580 ([M + NH_4]^+), 585 ([M + Na]^+). CD (MeOH): \( \theta \)_{289} +1.8×10^4, \( \theta \)_{279} +3.9×10^4, \( \theta \)_{234} -4.2×10^4, \( \theta \)_{272} -5.3×10^3. 1H-NMR (600 MHz, acetone-\( d_6 \), DMSO-\( d_6 \), 27 °C) δ: 2.56 (1H, m, H-3U), 2.61 (1H, dd, J = 8.5, 16.0 Hz, H-4a), 2.92 (2H, dd, J = 5.0, 16.0 Hz, H-4b, H-4c), 3.98 (1H, q, J = 6.5, J = 7.5, J = 8.5 Hz, H-3), 4.63 (1H, br s, H-2), 4.68 (1H, m, H-5), 4.75 (1H, m, H-6), 5.95 (2H, s, H-5, H-6), 6.10 (1H, s, H-6), 6.65 (1H, d, J = 8.5 Hz, H-5), 6.83 (1H, d, J = 2.0 Hz, H-5), 6.87 (1H, d, J = 2.0 Hz, H-2). 13C-NMR (150 MHz, acetone-\( d_6 \), DMSO-\( d_6 \), 27 °C) δ: 29.0 (C-4'), 30.1 (C-3'), 46.1 (C-1a), 68.3 (C-3), 76.8 (C-6'), 82.5 (C-2'), 96.1 (C-3', C-5'), 97.5 (C-6'), 100.5 (C-10'), 105.5 (C-1'), 107.2 (C-8'), 115.1 (C-2'), 115.5 (C-5'), 115.7 (C-5'), 116.7 (C-2'), 119.9 (C-6'), 120.7 (C-6'), 132.1 (C-1'), 135.5 (C-1'-), 143.6 (C-4'), 145.1 (C-3'), 145.4 (15-C-3'), 145.7 (C-9'), 153.5 (C-5'), 155.8 (C-7), 157.5 (C-4'), 157.8 (C-2'), 159.2 (C-6a).

Gambirin B1 (8): A brown amorphous powder, \( \text{IR} \) (KBr): 39.6° (e = 0.2, acetone).

References and Notes
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