[Chem. Pharm. Bull. 36(1) 39—47 (1988)]

Tannins and Related Compounds. LXI.[1] Isolation and Structures of Novel Bi- and Triflavanoids from the Leaves of *Cassia fistula* L.

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(Received June 11, 1987)

Together with (−)-epiafzelechin (**1**) and its 3-*O*-glucoside (**4**), (−)-epicatechin (**2**) and procyanidin B-2 (**3**), seven new biflavanoids (**5**—**7**, **9**—**12**) and two triflavanoids (**8**, **13**) have been isolated from the leaves of *Cassia fistula* L. (Leguminosae). On the basis of chemical and spectroscopic evidence, the structures of **5**—**8** were established to be proanthocyanidins each having (−)-epiafzelechin unit(s) in the molecule, while **9**—**13** were shown to consist of (2*S*)-7,4′-dihydroxyflavan and (−)-epiafzelechin units.

Keywords——*Cassia fistula*; Leguminosae; proanthocyanidin; flavan-3-ol glucoside; flavan-3-ol; flavan; condensed tannin; thiolytic degradation; acid-catalyzed condensation

The members of the family Leguminosae are known to produce a variety of phenolic metabolites with novel structures, especially flavonoids (*e.g.* peltogynoids, rotenoids, pterocarpans, coumestans, *etc.*). In contrast, there have been few reports on the chemistry of proanthocyanidins, except for Roux's notable work on those in the members of the genus *Acacia*.[2] As part of our chemical studies on polyphenolic constituents in the plants of the family Leguminosae, we have isolated ten new compounds **4**—**13**, together with (−)-epiafzelechin (**1**), (−)-epicatechin (**2**) and procyanidin B-2 (**3**), from the leaves of *Cassia fistula* L. This paper describes the isolation and structural elucidation of these compounds.

The fresh leaves of *Cassia fistula* collected in Taiwan were extracted with 80% aqueous acetone. The extract was subjected to a combination of Sephadex LH-20, MCI-gel CHP 20P, and Bondapak C_{18}/Porasil B chromatographies with various solvent systems to afford compounds **1**—**13**. Compounds **1**, **2** and **3** were identified as (−)-epiafzelechin,[3] (−)-epicatechin[4] and procyanidin B-2,[4] respectively, by comparisons of their physical data with those of authentic samples or with those described in the literature.

Compound **4** was obtained in a crystalline form (mp 234 °C). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **4** exhibited aliphatic proton signals at δ 5.05 (1H,

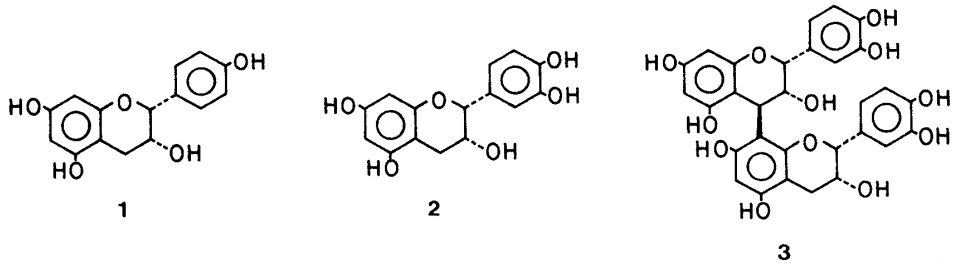
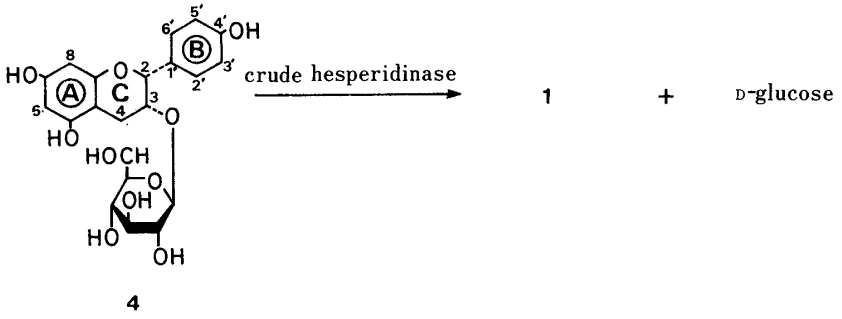


Chart 1

s), 4.31 (1H, m) and 2.90 (2H, m) arising from a flavan C-ring. The aromatic signals appeared as AB- and A_2B_2-type patterns, the former AB-signals being assigned to the flavan A-ring protons, and the latter to the B-ring ones. These ^{1}H-NMR data were consistent with those of (−)-epiafzelechin (**1**). In addition, the presence of a sugar moiety was revealed by the anomeric proton resonance [δ 4.19 (d, J=7 Hz)]. On enzymatic hydrolysis with crude hesperidinase, **4** afforded glucose and an aglycone which was identical with (−)-epiafzelechin (**1**). Glucose was concluded to be located at the C-3 hydroxyl group of the flavan moiety, since in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, the C-3 signal (δ 74.9) showed a downfield shift as compared with that (δ 66.9) of **1**. The configuration of the anomeric center was determined to be β on the basis of the coupling constant (J=7 Hz) of the anomeric proton signal. Accordingly, **4** was characterized as (−)-epiafzelechin 3-*O*-β-D-glucopyranoside.

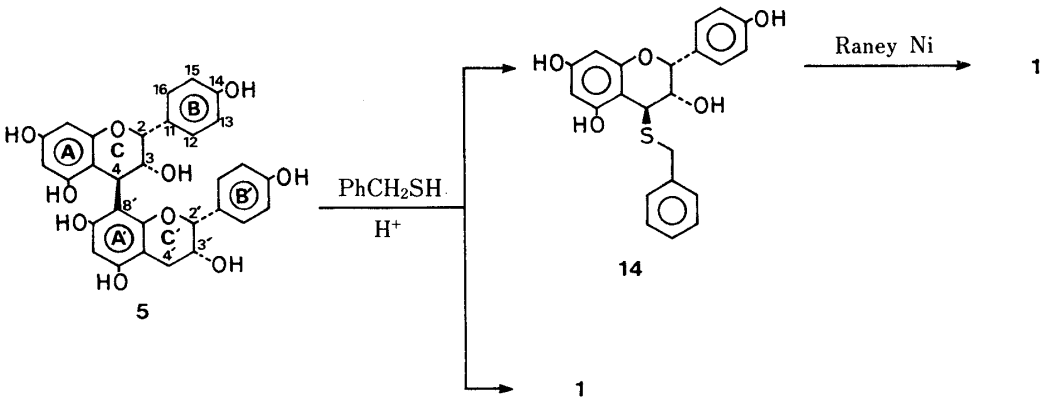


4

Chart 2

Compound **5** gave, with the anisaldehyde-sulfuric acid reagent, an orange-red coloration characteristic of proanthocyanidins. The occurrence of two flavan-3-ol units was deduced from the ^{13}C-NMR data (Table I). The chemical shifts (δ 79.3 and 77.0) of the flavan C-2 signals implied that the component units possess a 2,3-*cis* stereochemistry.[5] Furthermore, in the ^{1}H-NMR spectrum of **5**, the appearance of two A_2B_2-type signals attributed to the B-ring protons, as well as the coupling patterns [δ 5.02 and 5.11 (each s)] of the H-2 and H-2′ signals suggested that **5** consists entirely of epiafzelechin units. The structures of the component units were confirmed by thiolytic degradation. Treatment of **5** with benzylmercaptan in the presence of acid[6] gave **1** and a thioether (**14**). The thioether (**14**) was characterized as (−)-epiafzelechin 4-benzylthioether by ^{1}H-NMR analysis and by desulfurization with Raney nickel, giving **1**.

The location of the interflavanoid linkage was confirmed as follows. In the ^{1}H-NMR spectrum of **5**, the chemical shift (δ 5.02) of the lower H-2 signal was analogous to that (δ 4.96) of 4,8-linked procyanidin B-2 (**3**) rather than that (δ 4.84) of 4,6-linked procyanidin B-5 (**15**),



5

Chart 3

indicating that the component units are joined through a 4,8-interflavanoid linkage.[7] The configuration of the interflavanoid linkage was determined to be β on the basis of the coupling constant ($J \fallingdotseq 0$ Hz) of the H-4 signal.

From these spectral and chemical findings, **5** was characterized as epiafzelechin-(4β→8)-epiafzelechin.

The negative fast atom bombardment mass spectra (FAB-MS) of compounds **6** and **7** showed the same $(M-H)^-$ ion peak at m/z 561. The ^{1}H-NMR spectra of **6** and **7** were almost indistinguishable from each other, and also were closely similar to that of **5**, except for the aromatic signals. In addition to these findings, the appearance of A_2B_2- and ABX-type aromatic signals in both spectra suggested the occurrence of the epiafzelechin and epicatechin units. In order to confirm the structures of the component units, similar thiolytic degradation was attempted. On treatment with benzylmercaptan containing acid, **6** afforded (−)-epiafzelechin 4-benzylthioether (**14**) and **2**, while **7** yielded (−)-epicatechin 4-benzylthioether (**16**) and **1**.

The location and the configuration of the interflavanoid linkage were concluded to be C(4β)–C(8) in each case on the basis of the chemical shifts (δ 4.95 in **6** and δ 4.96 in **7**) of the lower H-2 signals and the coupling patterns (δ 4.75, $J \fallingdotseq 0$ Hz in **6** and δ 4.72, $J \fallingdotseq 0$ Hz in **7**) of

TABLE I. ^{13}C-NMR Data for Compounds **5**—**8**[a]

	5	6	7	8
C-2	77.0	76.9	77.0	77.0
C-3	72.5	72.8	72.5	72.1
C-4	36.7	36.9	36.7	36.7
C-2′	79.3	79.2	79.4	77.0
C-3′	66.7	66.3	66.8	72.1
C-4′	29.4	—[b]	—[b]	36.7
C-2″				79.4
C-3″				66.4
C-4″				—[b]

a) Measured in acetone-d_6 + D_2O at 25.05 MHz. *b*) Overlapped with solvent signals.

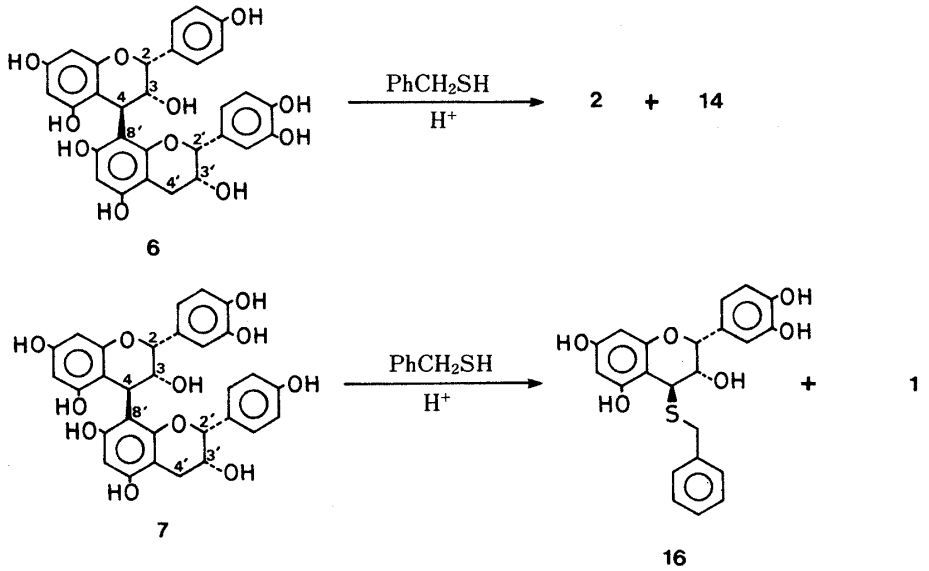


Chart 4

the upper H-4 signals.

Accordingly, the structures of **6** and **7** were established to be epiafzelechin-(4β→8)-epicatechin and epicatechin-(4β→8)-epiafzelechin, respectively.

The triflavanoid constitution of compound **8** was shown by FAB-MS analysis [*m/z*: 817 $(M-H)^-$]. The occurrence of three epiafzelechin units in the molecule was deduced from the ^{1}H-NMR (see experimental and ^{13}C-NMR data (Table I). This was also supported by the result of thiolytic degradation, giving **14** and **1**.

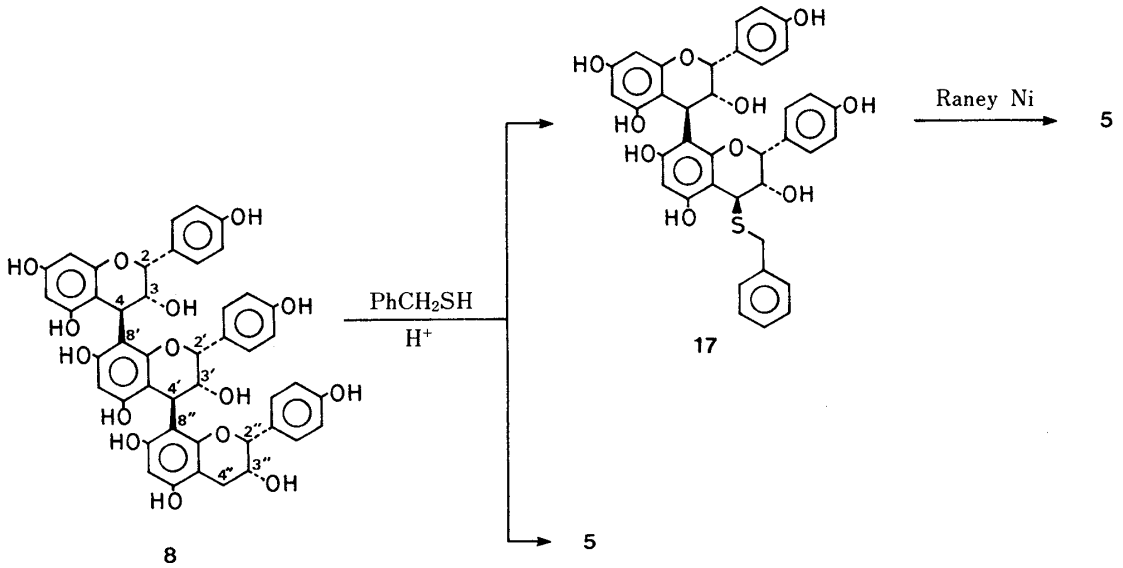


Chart 5

The locations and the configurations of the linkages between the component units were determined as follows. Partial thiolytic degradation of **8** afforded, together with **14** and **1**, **5** and a thioether (**17**) which was characterized as the 4′-benzylthioether of **5** by its conversion with Raney nickel to **5**. Among these degradation products, the formation of **5** and **17** indicated that all component units are linked through 4β,8-interflavanoid linkages. Thus, **8** was concluded to be epiafzelechin-(4β→8)-epiafzelechin-(4β→8)-epiafzelechin.

The FAB-MS of compounds **9** and **10** gave the same $(M-H)^-$ ion peak at *m/z* 513, corresponding to a biflavanoid constitution, although each molecular weight was 32 mass units less than that of **5**.

The ^{13}C-NMR spectrum of **9** showed six aliphatic signals ascribable to the flavan C-ring carbons. The chemical shifts (δ 78.9, 65.6 and 29.5) of three of these signals were consistent with those in the flavan-3-ol framework. The remaining three signals (δ 76.0, 34.8 and 27.8) were assigned to flavan C-2, C-3 and C-4 carbons, respectively, and the chemical shift of the C-3 signal indicated the absence of a hydroxyl group at this position. An attempt to obtain a thioether from the upper unit by thiolytic degradation was unsuccessful. However, acid-catalyzed degradation of **9** with 1 N ethanolic HCl furnished, along with uncharacterized red pigments, **1** which had arisen from the lower unit. The upper unit of **9** was shown to possess a 7,4′-dihydroxyflavan framework, since the ^{1}H-NMR spectrum showed ABX- and A_2B_2-type aromatic signals arising from the upper unit and the coupling patterns were consistent with those of the A- and B-ring protons of liquiritigenin (7,4′-dihydroxyflavanone) (**18**). Based on these chemical and spectroscopic findings, **9** was concluded to consist of 7,4′-dihydroxyflavan and (−)-epiafzelechin units.

On the other hand, the ^{13}C-NMR spectrum of **10**, measured at room temperature, was complicated by conformational isomerism.[6] However, the signals arising from the major

conformer could be assigned. The chemical shifts (δ 79.6, 78.5, 65.4, 36.6, 32.5 and 29.7) of the C,C′-ring carbon signals suggested the occurrence of flavan and flavan-3-ol units in the molecule, while the aromatic signal patterns were closely similar to those in **9**. These data, in conjunction with the fact that similar acid-catalyzed degradation of **10** afforded **1**, implied that **10** also consists of 7,4′-dihydroxyflavan and (−)-epiafzelechin units.

The units of 7,4′-dihydroxyflavan and (−)-epiafzelechin in **9** and **10** were considered to be linked between the C-4 and C-8 (or C-6) positions in each case from the ^{13}C-NMR splitting patterns of the upper C-4 [δ 27.8 in **9** and δ 32.5 in **10** (each d)] and lower C-8 (or C-6) carbons [δ 107.2 in **9** and δ 106.6 in **10** (each s)]. Further confirmation of the positions of the interflavanoid linkages was achieved by analyses of the ^{1}H-NMR spectra of **9** and **10**. In the spectra, the lower H-2 signals (δ 4.72 in **9** and δ 4.48 in **10**) were observed at higher field than that (δ 4.91) of (−)-epiafzelechin. This implied that the H-2 proton of the lower unit is magnetically affected by the aromatic ring in the upper unit, indicating that the component units in **9** and **10** are joined through a 4,8-linkage.[8] The configuration of the interflavanoid linkage in each compound was deduced on the basis of the ^{13}C-NMR chemical shift. It has been reported that the upper C-2 signal in a dimeric proanthocyanidin possessing a β-interflavanoid linkage appeared upfield by 2—3 ppm as compared with that in the α-isomer.[5] The upper C-2 signals in **9** and **10** were observed at δ 76.0 and 79.6, thus indicating the configurations of the interflavanoid linkages in **9** and **10** to be β and α, respectively.

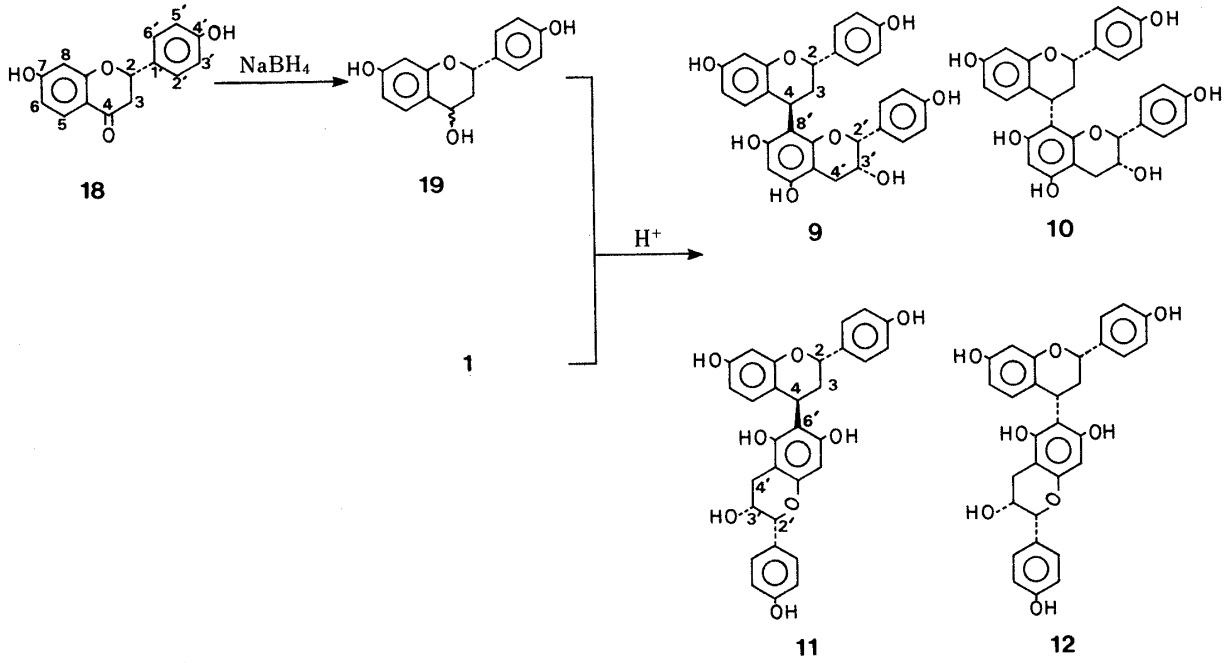


Chart 6

Accordingly, **9** and **10** were concluded to be 7,4′-dihydroxyflavan-(4β→8)-epiafzelechin and 7,4′-dihydroxyflavan-(4α→8)-epiafzelechin, respectively.

Compounds **11** and **12** exhibited the same $(M-H)^-$ ion peak at m/z 513 as those of **9** and **10** in the negative FAB-MS. The ^{13}C-NMR spectra of **11** and **12** were closely similar to those of **9** and **10**, respectively, and showed the presence of 7,4′-dihydroxyflavan and epiafzelechin units in each molecule.

The locations and the configurations of the interflavanoid linkages were determined as follows. In the ^{1}H-NMR spectra of **11** and **12**, the chemical shifts (δ 4.89 in **11** and 4.90 in **12**) of the lower H-2 signals were consistent with that (δ 4.91) in (−)-epiafzelechin (**1**), thus indicating that the lower unit was less affected by the upper unit in each case. Consequently,

the component units in **11** and **12** were concluded to be joined through 4,6-interflavanoid linkages. The configurations at the C-4 positions in **11** and **12** were determined to be β and α, respectively on the basis of the ^{13}C-NMR chemical shifts (δ 76.0 in **11** and δ 79.0—80.0 in **12**) of the upper C-2 signals.

In order to confirm the structures of **9**—**12** including the absolute stereochemistry, preparation of these compounds from compounds with the known stereochemistry was attempted. (−)-Liquiritigenin (**18**) was treated with $NaBH_4$ to afford a compound, which was characterized as (2*S*)-7,4′-dihydroxyflavan-4-ol (**19**) by ^{1}H- and ^{13}C-NMR analyses. On acid treatment, (2*S*)-7,4′-dihydroxyflavan-4-ol (**19**) generated the 4-carbocation with retention of the configuration at the C-2 position.[9] In the presence of (−)-epiafzelechin (**1**), this carbocation attacked the C-6 and C-8 positions of **1** to furnish four compounds which were shown to be identical with **9**, **10**, **11** and **12** by comparisons of the physical and spectral data.

On the basis of these spectral and chemical findings, compounds **9**, **10**, **11** and **12** were characterized as (2*S*)-7,4′-dihydroxyflavan-(4β→8)-epiafzelechin, (2*S*)-7,4′-dihydroxyflavan-(4α→8)-epiafzelechin, (2*S*)-7,4′-dihydroxyflavan-(4β→6)-epiafzelechin and (2*S*)-7,4′-dihydroxyflavan-(4α→6)-epiafzelechin, respectively.

Compound **13** exhibited the $(M-H)^-$ ion peak at *m/z* 785 in the negative FAB-MS. The ^{13}C-NMR spectrum of **13** showed, together with signal patterns similar to those of **9**, additional signals arising from an epicatechin unit, thus suggesting the occurrence of one 7,4′-dihydroxyflavan moiety and two epicatechin units in the molecule.

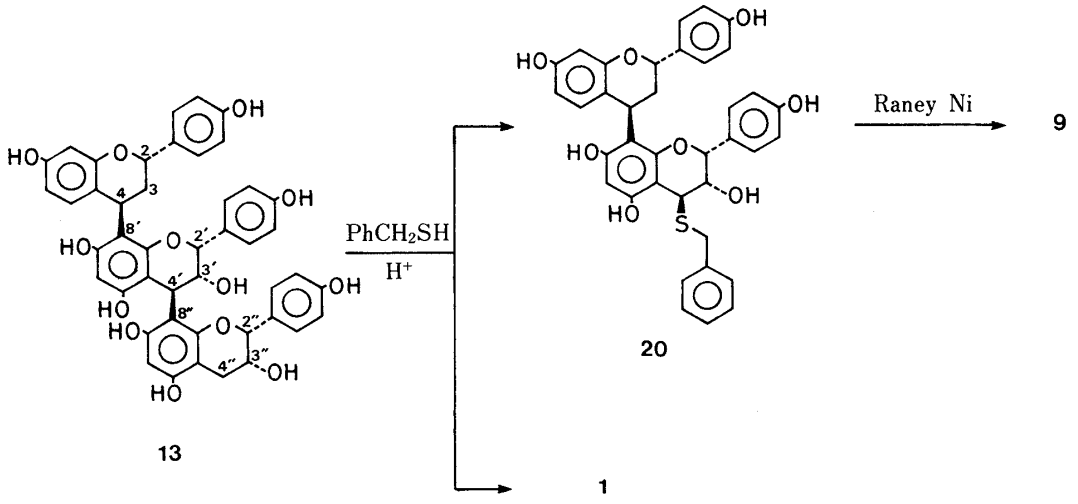


Chart 7

Application of thiolytic degradation to **13** resulted in selective cleavage of the interflavanoid linkage between the lower two units to afford **1** and a thioether (**20**). This thioether was characterized as the 4′-benzylthioether of **9** by ^{1}H-NMR analysis and by its conversion with Raney nickel to **9**. Accordingly, the component units were concluded to be linked in the sequence of (2*S*)-7,4′-dihydroxyflavan-(4β→8)-epiafzelechin-(4→6 or 4→8)-epiafzelechin. The interflavanoid linkage between the lower two units was presumed to be at the C(4) and C(8) positions, since in the ^{1}H-NMR spectrum of **13**, the chemical shift (δ 5.04) of the H-2″ signal was analogous to that (δ 5.06) of **8**. In order to determine the location of the lower interflavanoid linkage, a similar condensation reaction was attempted. Treatment of (2*S*)-7,4′-dihydroxyflavan-4-ol (**19**) and **5** in 1 N ethanolic HCl gave several products, of which the major product was found to be identical with **13**.

Based on these spectral and chemical data, **13** was characterized as (2*S*)-7,4′-dihydroxyflavan-(4β→8)-epiafzelechin-(4β→8)-epiafzelechin.

Experimental

Details of the instruments and chromatographic conditions used throughout this work were the same as described in the previous paper.[1)]

Extraction and Isolation——The fresh leaves of *Cassia fistula* (collected in Taiwan) were extracted with 80% aqueous acetone. The acetone was removed by evaporation under reduced pressure, and the resulting aqueous solution was shaken with $CHCl_3$ to remove chlorophylls, waxes, *etc.* The water-soluble portion was concentrated to dryness under reduced pressure, the residue was applied to a column of Sephadex LH-20, and elution with EtOH gave three fractions; fr. 1 (12 g), fr. 2 (38 g) and fr. 3 (6 g). Fr. 1 was rechromatographed over Sephadex LH-20 with 60% aqueous MeOH and MCI-gel CHP 20P with 30% aqueous MeOH to furnish compounds **4** (52 mg), **1** (2.5 g) and **2** (1.1 g). Fr. 2 gave, on chromatography over Sephadex LH-20 with 60% aqueous MeOH, three further fractions (fr. 2a, fr. 2b and fr. 2c). Fr. 2a was rechromatographed on MCI-gel CHP 20P (30% aqueous MeOH) and Bondapak C_{18} to furnish compounds **5** (2.5 g), **6** (62 mg), **7** (28 mg) and **3** (18 mg). Separation of fr. 2b by repeated chromatography over Sephadex LH-20 (60% aqueous MeOH) and Bondapak C_{18} (30% aqueous MeOH) afforded compounds **9** (3.5 g) and **10** (1.8 g), while fr. 2c was subjected to Bondapak C_{18} chromatography (30% aqueous MeOH) to afford compounds **11** (28 mg) and **12** (13 mg). Rechromatography of fr. 3 on MCI-gel CHP 20P with 40% aqueous MeOH gave compounds **8** (282 mg) and **13** (110 mg).

Compound 4——Colorless needles, mp 234 °C, $[\alpha]_D^{18}$ −56.3° ($c=1.1$, MeOH). Negative FAB-MS *m/z*: 435 $(M-H)^-$. *Anal.* Calcd for $C_{21}H_{24}O_{10}\cdot H_2O$: C, 55.50; H, 5.77. Found: C, 55.91; H, 5.32. ^{1}H-NMR (acetone-d_6) δ: 2.90 (2H, m, H-4), 4.19 (1H, d, $J=7$ Hz, anomeric H), 4.31 (1H, m, H-3), 5.05 (1H, s, H-2), 5.91, 6.02 (each 1H, d, $J=2$ Hz, H-6, 8), 6.78 (2H, d, $J=8$ Hz, H-3′, 5′), 7.38 (2H, d, $J=8$ Hz, H-2′, 6′). ^{13}C-NMR (acetone-d_6) δ: 24.7 (C-4), 62.9 (glc C-6), 71.4 (glc C-4), 74.9 (C-3), 75.4 (glc C-2), 77.3, 77.5 (glc C-3, 5), 78.1 (C-2), 95.6, 96.4 (C-6, 8), 100.0 (C-4a), 104.7 (glc C-1), 115.6 (C-3′, 5′), 129.2 (C-2′, 6′), 130.6 (C-1′), 156.8, 157.3, 157.6 (C-5, 7, 8a).

Enzymatic Hydrolysis of 4 with Crude Hesperidinase——A solution of **4** (30 mg) in H_2O (5 ml) was incubated overnight with crude hesperidinase at 37 °C. The solvent was evaporated off under reduced pressure, and the residue was treated with MeOH. The MeOH-soluble portion was subjected to Sephadex LH-20 chromatography. Elution with MeOH afforded glucose [*Rf*: 0.38; solvent: *n*-BuOH–pyridine–H_2O (6 : 4 : 3)] and (−)-epiafzelechin (**1**) (8 mg).

Compound 5——Colorless prisms, mp 250 °C, $[\alpha]_D^{23}$ +42.3° ($c=0.7$, MeOH). Negative FAB-MS *m/z*: 545 $(M-H)^-$. *Anal.* Calcd for $C_{30}H_{26}O_{10}\cdot 1.5H_2O$: C, 62.82; H, 5.10. Found: C, 62.51; H, 4.88. ^{1}H-NMR (acetone-d_6) δ: 2.90 (2H, m, H-4′), 4.23 (2H, br s, H-3, 3′), 4.72 (1H, s, H-4), 5.02 (1H, s, H-2′), 5.11 (1H, s, H-2), 5.80—6.10 (3H in total, m, A-ring H), 6.72, 6.77 (each 2H, d, $J=8$ Hz, H-13, 13′, 15, 15′), 7.20 (4H, d, $J=8$ Hz, H-12, 12′, 16, 16′).

Thiolytic Degradation of 5——A mixture of **5** (50 mg), benzylmercaptan (1.5 ml) and acetic acid (1 ml) in EtOH (10 ml) was refluxed for 10 h with stirring. The reaction mixture was concentrated under reduced pressure, and the oily residue was chromatographed over Sephadex LH-20 (EtOH), affording (−)-epiafzelechin (**1**) (8 mg) and a thioether (**14**) (11 mg). **14**: An off-white amorphous powder, $[\alpha]_D^{18}$ −38.3° ($c=1.6$, MeOH). *Anal.* Calcd for $C_{22}H_{20}O_5S\cdot H_2O$: C, 63.75; H, 5.35: Found: C, 63.59; H, 5.00. ^{1}H-NMR (acetone-d_6) δ: 3.99 (1H, m, H-3), 4.01 (2H, s, $-SCH_2$), 4.12 (1H, d, $J=2$ Hz, H-4), 5.32 (1H, s, H-2), 5.90, 6.04 (each, d, $J=2$ Hz, H-6, 8), 6.82 (2H, d, $J=8$ Hz, H-13, 15), 7.33 (2H, d, $J=8$ Hz, H-12, 16), 7.10—7.60 (5H in total, m, aromatic H).

Desulfurization of 14——The thioether (**14**) (10 mg) in EtOH–acetic acid (9 : 1) (1 ml) was shaken at 50 °C with Raney nickel (W-4) for 30 min. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Sephadex LH-20 chromatography (60% aqueous MeOH) to give (−)-epiafzelechin (**1**) (5 mg).

Compound 6——An off-white amorphous powder, $[\alpha]_D^{18}$ +42.3° ($c=1.3$, MeOH). Negative FAB-MS *m/z*: 561 $(M-H)^-$. *Anal.* Calcd for $C_{30}H_{26}O_{11}\cdot 2H_2O$: C, 60.20; H, 5.05. Found: C, 60.14; H, 4.95. ^{1}H-NMR (acetone-d_6) δ: 2.82 (2H, m, H-4′), 4.03 (1H, s, H-3), 4.30 (1H, br s, H-3′), 4.75 (1H, s, H-4), 4.95 (1H, s, H-2′), 5.17 (1H, s, H-2), 5.90—6.10 (3H in total, m, A-ring H), 6.69 (1H, d, $J=8$ Hz, H-15′), 6.75 (2H, d, $J=8$ Hz, H-13, 15), 6.87 (1H, dd, $J=8$, 1 Hz, H-16′), 7.08 (1H, br s, H-2′), 7.26 (2H, d, $J=8$ Hz, H-12, 16).

Thiolytic Degradation of 6——A mixture of **6** (50 mg), benzylmercaptan (1 ml) and acetic acid (1 ml) in EtOH (10 ml) was heated under reflux for 8 h with stirring. Work-up as described for **5** gave (−)-epiafzelechin 4-benzylthioether (**14**) (25 mg) and (−)-epicatechin (**2**) (12 mg).

Compound 7——An off-white amorphous powder, $[\alpha]_D^{20}$ +32.3° ($c=0.6$, MeOH). Negative FAB-MS *m/z*: 561 $(M-H)^-$. *Anal.* Calcd for $C_{30}H_{26}O_{11}\cdot 2H_2O$: C, 60.20; H, 5.05. Found: C, 60.53; H, 4.77. ^{1}H-NMR (acetone-d_6) δ: 2.84 (2H, m, H-4′), 4.23 (2H, br s, H-3, 3′), 4.72 (1H, s, H-4), 4.96 (1H, s, H-2′), 5.11 (1H, s, H-2), 5.80—6.10 (3H, in total, m, A-ring H), 6.76 (2H, d, $J=8$ Hz, H-13′, 15′), 6.80—6.90 (2H in total, m, H-15, 16), 7.04 (1H, br s, H-12), 7.28 (2H, d, $J=8$ Hz, H-12′, 16′).

Thiolytic Degradation of 7——A mixture of **7** (20 mg), benzylmercaptan (1 ml) and acetic acid (1 ml) in EtOH (7 ml) was treated as described for **6**. The reaction products were separated as described above to give (−)-epicatechin 4-benzylthioether (**16**) (8 mg) and (−)-epiafzelechin (**1**) (5 mg).

Compound 8——An off-white amorphous powder, $[\alpha]_D^{20}$ +51.3° ($c=1.1$, MeOH). Negative FAB-MS *m/z*: 817 $(M-H)^-$. *Anal.* Calcd for $C_{45}H_{38}O_{15}\cdot 3H_2O$: C, 61.92; H, 5.08. Found: C, 62.33; H, 4.91. ^{1}H-NMR (acetone-d_6) δ:

2.84 (2H, m, H-4″), 4.02 (1H, br s, H-3′), 4.33 (2H, br s, H-3, 3″), 4.80 (2H, s, H-4, 4′), 5.08, 5.20, 5.24 (each 1H, s, H-2, 2′, 2″), 5.80—6.10 (4H in total, m, A-ring H), 6.40—7.60 (12H in total, m, B-ring H).

Thiolytic Degradation of 8——A mixture of **8** (50 mg), benzylmercaptan (1.5 ml) and acetic acid (1 ml) in EtOH (10 ml) was refluxed for 13 h. The reaction mixture was worked up as before to yield (−)-epiafzelechin 4-benzylthioether (**14**) (25 mg) and (−)-epiafzelechin (**1**) (8 mg).

Partial Thiolytic Degradation of 8——A mixture of **8** (100 mg), benzylmercaptan (1.5 ml) and acetic acid (1 ml) in EtOH (10 ml) was heated under reflux for 6 h with stirring. The reaction mixture was concentrated under reduced pressure to give an oily residue, which was repeatedly chromatographed over Sephadex LH-20 (EtOH) and MCI-gel CHP 20P (40% aqueous MeOH) to give (−)-epiafzelechin (**1**) (5 mg), (−)-epiafzelechin 4-benzylthioether (**14**) (10 mg), **5** (14 mg) and a thioether (**17**) (10 mg). **17**: An off-white amorphous powder, $[\alpha]_D^{18}$ +64.5° (c=1.1, acetone). *Anal.* Calcd for $C_{37}H_{32}O_{10}S \cdot H_2O$: C, 64.71; H, 4.99. Found: C, 64.81; H, 4.50. ^{1}H-NMR (acetone-d_6) δ: 3.93 (1H, br s, H-3). 4.07 (2H, s, $-SCH_2-$), 4.20 (1H, br s, H-3′), 4.23 (1H, d, J=2 Hz, H-4′), 4.72 (1H, s, H-4), 5.09, 5.18 (each 1H, s, H-2), 5.80—6.10 (3H in total, A-ring H), 6.40—7.80 (9H in total, B-ring H, aromatic H).

Desulfurization of 17——The thioether (**17**) (10 mg) in EtOH was treated with Raney nickel at 50 °C for 30 min. The reaction mixture was worked up as before to furnish **5** (3 mg).

Compound 9——Colorless prisms, mp 213—215 °C, $[\alpha]_D^{20}$ +102.3° (c=1.5, MeOH). Negative FAB-MS m/z: 513 $(M-H)^-$. *Anal.* Calcd for $C_{30}H_{26}O_8 \cdot H_2O$: C, 67.66; H, 5.30. Found: C, 67.85; H, 50.2 ^{1}H-NMR (acetone-d_6) δ: 2.00—2.80 (2H, m, H-3), 2.84 (2H, m, H-4′), 4.30 (1H, m, H-3′), 4.51 (1H, t, J=8 Hz, H-4), 4.72 (1H, s, H-2′), 5.38 (1H, dd, H-2), 6.07 (1H, s, H-6′), 6.19 (1H, dd, J=8, 2 Hz, H-6), 6.30 (1H, d, J=2 Hz, H-8), 6.63 (1H, d, J=8 Hz, H-5), 6.72 (4H, d, J=8 Hz, H-13, 13′, 15, 15′), 7.12, 7.13 (each 2H, d, J=8 Hz, H-12, 12′, 16, 16′). ^{13}C-NMR (acetone-d_6) δ: 27.8 (d, C-4), 29.5 (t, C-4′), 34.8 (t, C-3), 65.6 (d, C-3′), 76.0 (d, C-2), 78.9 (d, C-2′), 96.6 (d, C-6′), 99.9 (s, C-10′), 103.7 (d, C-8), 108.4 (d, C-6), 109.9 (s, C-8′).

Acid-Catalyzed Degradation of 9——A solution of **9** (50 mg) in 1 N ethanolic HCl (10 ml) was refluxed for 1 h with stirring. The solvent was evaporated off under reduced pressure, and the residue was chromatographed on a column of Sephadex LH-20. Elution with 60% aqueous MeOH furnished (−)-epiafzelechin (**1**) (15 mg).

Compound 10——Colorless prisms, mp 211 °C, $[\alpha]_D^{20}$ −88.3° (c=1.5, MeOH). Negative FAB-MS m/z: 513 $(M-H)^-$. *Anal.* Calcd for $C_{30}H_{26}O_8 \cdot H_2O$: C, 67.66; H, 5.30. Found: C, 67.56; H, 5.11. ^{1}H-NMR (acetone-d_6) δ: 1.80—3.00 (4H in total, m, H-3′, 4′), 4.28 (1H, m, H-3′), 4.48 (1H, s, H-2), 4.83 (1H, dd, J=10, 6 Hz, H-4), 5.05 (1H, dd, J=9, 2 Hz, H-2), 6.00—7.50 (12H in total, m, A, A′, B, B′-ring H). ^{13}C-NMR (acetone-d_6) δ: 29.4, 29.7 (each t, C-4′), 32.5 (d, C-4), 36.6 (t, C-3), 65.4, 66.6 (d, C-3′), 78.5 (d, C-2′), 79.6 (d, C-2), 96.0, 97.2 (each d, C-6′), 99.5, 101.3 (each s, C-10′), 103.5 (d, C-8), 108.4, 108.8 (each d, C-6), 109.9 (s, C-8′).

Compound 11——Colorless prisms, mp 221 °C, $[\alpha]_D^{18}$ +56.3° (c=1.5, MeOH). Negative FAB-MS m/z: 513 $(M-H)^-$. *Anal.* Calcd for $C_{30}H_{26}O_8 \cdot H_2O$: C, 67.66; H, 5.30. Found: C, 67.89; H, 4.99. ^{1}H-NMR (acetone-d_6) δ: 2.00—3.00 (4H, in total, m, H-3, 4′), 4.20 (1H, br s, H-3′), 4.49 (1H, t, J=6 Hz, H-4), 4.89 (1H, s, H-2′), 5.35 (1H, dd, J=8, 4 Hz, H-2), 6.03 (1H, s, H-8), 6.30 (1H, dd, J=8, 2 Hz, H-5), 6.77, 6.78 (each 2H, d, J=8 Hz, H-13, 13′, 15, 15′), 7.19, 7.34 (each 2H, d, J=8 Hz, H-12, 12′, 16, 16′). ^{13}C-NMR (acetone-d_6) δ: 35.5 (C-3), 66.8 (C-3′), 76.0 (C-2), 79.3 (C-2′), 96.6 (C-8′), 101.4 (C-10′), 104.0 (C-8), 109.2 (C-6, 6′).

Compound 12——Colorless prisms, mp 228 °C, $[\alpha]_D^{20}$ −50.8° (c=1.5, MeOH). Negative FAB-MS m/z: 513 $(M-H)^-$. *Anal.* Calcd for $C_{30}H_{26}O_8 \cdot H_2O$: C, 67.66; H, 5.30. Found: C, 67.57; H, 4.85. ^{1}H-NMR (acetone-d_6) δ: 2.80—3.00 (4H in total, m, H-3, 4′), 4.24 (1H, br s, H-3′), 4.84 (1H, d-like J=10 Hz, H-4), 4.90 (1H, s, H-2′), 5.09 (1H, d-like, J=12 Hz, H-2), 5.80—7.60 (12H in total, m, A, A′, B, B′-ring H). ^{13}C-NMR (acetone-d_6) δ: 32.4, 33.3 (C-4), 36.3, 36.5 (C-3), 66.7, 66.8 (C-3′), 79.2, 79.4, 79.5, 79.6 (C-2, 2′), 96.1, 97.5 (C-8′), 100.3 (C-10′), 103.7, 104.2 (C-8), 108.6, 109.4 (C-6, 6′).

Reduction of (−)-Liquiritigenin (18)——A solution of **18** (300 mg) in MeOH (10 ml) was treated with $NaBH_4$ at room temperature for 5 min. The reaction mixture was neutralized with Amberlite IR 120B (H^+ form), and the solution was concentrated to dryness under pressure. The residue was subjected to Sephadex LH-20 chromatography with acetone to afford **19** (210 mg). **19**: Colorless needles, $[\alpha]_D^{20}$ −48.5° (c=1.1, MeOH). FAB-MS m/z: 257 $(M-H)^-$. ^{1}H-NMR (acetone-d_6) δ: 1.80—2.50 (2H, m, H-3), 4.96 (1H, dd, J=10, 6 Hz, H-4), 5.08 (1H, dd, J=12, 2 Hz, H-2), 6.11 (1H, d, J=2 Hz, H-8), 6.39 (1H, dd, J=8, 2 Hz, H-6), 6.83 (2H, d, J=8 Hz, H-13, 15), 7.31 (2H, d, J=8 Hz, H-12, 16), 7.33 (1H, d, J=8 Hz, H-5).

Preparation of 9, 10, 11 and 12——A mixture of **1** (180 mg) and **19** (210 mg) in 1 N ethanolic HCl (50 ml) was shaken for 10 min at room temperature. The reaction mixture was applied to a column of Sephadex LH-20, pre-swollen in H_2O. After washing of the column with H_2O, elution with 80% aqueous MeOH afforded two fractions, which were separately subjected to repeated chromatographies over Sephadex LH-20 (EtOH) and Bondapak C_{18} (30% aqueous MeOH) to give **9** (51 mg), **10** (21 mg), **11** (7 mg) and **12** (6 mg).

Compound 13——Colorless prisms, mp 221 °C, $[\alpha]_D^{23}$ +107.3° (c=1.5, MeOH). Negative FAB-MS m/z: 785 $(M-H)^-$. *Anal.* Calcd for $C_{45}H_{38}O_{13} \cdot 3H_2O$: C, 64.28; H, 5.27. Found: C, 64.00; H, 5.21. ^{1}H-NMR (acetone-d_6) δ: 2.00—3.00 (4H in total, m, H-3, 4″), 4.36 (2H, br s, H-3′, 3″), 4.40—4.80 (2H in total, m, H-4, 4′), 4.93, 5.04 (each 2H, s, H-2′, 2″), 5.40 (1H, m, H-2′), 5.80—7.60 (17H in total, m, A, A′, A″, B′, B″-ring H). ^{13}C-NMR (acetone-d_6) δ: 34.2 (C-3), 37.0 (C-4′), 66.3 (C-3″), 70.7 (C-3′), 76.1 (C-2, 2′), 79.3 (C-2″), 96.6, 96.9 (C-6′, 6″), 100.3 (C-10′,

10″), 103.4 (C-8), 107.2, 108.5 (C-6), 110.5 (C-6).

Thiolytic Degradation of 13——A mixture of **13** (50 mg), benzylmercaptan (1 ml) and acetic acid (1 ml) in EtOH (10 ml) was heated under reflux for 8 h with stirring. The reaction mixture was treated as before to give (−)-epiafzelechin (**1**) (8 mg) and the thioether **20** (20 mg). **20**: An off-white amorphous powder, $[\alpha]_D^{23}$ +28.5° (c=1.1, MeOH). *Anal.* Calcd for $C_{37}H_{32}O_8S \cdot H_2O$: C, 67.87; H, 4.88. Found: C, 68.20; H, 4.88. ^{1}H-NMR (acetone-d_6) δ: 2.00—2.90 (2H in total, m, H-3), 4.01 (2H, s, $-SCH_2$), 4.08, 4.15 (each 1H, d, J=2 Hz, H-3′, 4′), 4.50 (1H, t, J=7 Hz, H-4), 5.12 (1H, s, H-2′), 5.40 (1H, t, J=5 Hz, H-2), 6.13 (1H, s, H-6′), 6.18 (1H, dd, J=8, 2 Hz, H-6), 6.31 (1H, d, J=2 Hz, H-8), 6.62 (1H, d, J=8 Hz, H-5), 6.72, 6.73 (each 2H, d, J=8 Hz, H-13, 13′, 15, 15′), 6.90—7.60 (9H in total, m, H-12, 12′, 13, 13′).

Desulfurization of 20——The thioether **20** (20 mg) in EtOH–acetic acid (9:1) (10 ml) was treated with Raney nickel at 50 °C for 30 min. Work-up as described for **8** furnished **9** (9 mg).

Preparation of 13——A solution of **19** (50 mg) and **5** (50 mg) in 1 N ethanolic HCl was shaken for 5 min at room temperature. The reaction mixture was worked up as before to give **13** (24 mg).

Acknowledgement The authors wish to thank Mr. Y. Tanaka, Miss K. Soeda and Mr. R. Isobe for the measurements of negative FAB-MS, and ^{1}H- and ^{13}C-NMR spectra, and the staff of the Central Analysis Room of this university for elemental analysis.

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