Tannins and Related Compounds. XCIII.[1] Occurrence of Enantiomeric Proanthocyanidins in the Leguminosae Plants, *Cassia fistula* L. and *C. javanica* L.

Yoshiki KASHIWADA, Hiroyuki IIZUKA, Keiko YOSHIOKA, Rong-Fu CHEN, Gen-ichiro NONAKA and Itsuo NISHIOKA*

Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan. *Received August 29, 1989*

Proanthocyanidins containing flavan-3-ol (epiafzelechin and epicatechin) units with an abnormal 2*S*-configuration have been isolated from the pods of *Cassia fistula* L. and the bark of *C. javanica* L. (Leguminosae), together with common flavan-3-ols and proanthocyanidins. The structures of these compounds have been established on the basis of chemical and spectroscopic evidence. In addition, the flavan-3-ols and proanthocyanidins obtained from *C. fistula* have been found to be partly racemic, and the proportions of each enantiomer have been determined by high-performance liquid chromatography using an optical resolution column.

Keywords *Cassia fistula*; *Cassia javanica*; Leguminosae; enantiomerism; enantiomeric proanthocyanidin; 2*S*-flavan-3-ol; (+)-epiafzelechin; (+)-epicatechin; chiral resolution HPLC

Proanthocyanidins consist of various flavan-3-ol units, *e.g.* afzelechin, epiafzelechin, catechin, epicatechin, gallocatechin and epigallocatechin, normally possessing a 2*R*-configuration. In contrast, the existence of proanthocyanidins containing a flavan-3-ol with 2*S*-configuration as a component unit has so far been reported to be limited to the Monocotyledonae (Arecidae, Commelinidae and Lillidae)[2,3] and a single species (*Ephedra sinica* L.) of the Gymnospermae.[4a] In the course of our chemical examinations on polyphenolic constituents in the plants of the family Leguminosae, we have found that the proanthocyanidins in *Cassia fistula* L. and *C. javanica* L. contain flavan-3-ol units with 2*S*-configuration, and that flavan-3-ols and proanthocyanidins isolated from *C. fistula* are partly racemic. This paper deals with the isolation and characterization of these proanthocyanidins, and also describes the determination of the ratio of 2*R*:2*S* units by application of chiral resolution high-performance liquid chromatography (HPLC).

The fresh pods of *C. fistula*, collected in Taiwan, were extracted with 80% aqueous acetone, and the extract was subjected to a combination of Sephadex LH-20, MCI-gel CHP 20P, Bondapak C_{18}/Porasil B and Kusano packed column octadesyl silica (ODS) chromatographies with various solvent systems to yield eleven compounds, tentatively designated as CF-1—CF-11. Similar extraction and chromatographies of dried bark of *C. javanica*, collected in Taiwan, afforded seven compounds, named CJ-1—CJ-7. Among these compounds, CF-1 and -2, and CJ-1 and -2 were found to be identical with (+)-catechin (**1**),[5] (+)-epiafzelechin (**2b**),[3a] (−)-epiafzelechin (**2a**)[6] and (−)-epicatechin (**3a**),[7] respectively.

The proton-nuclear magnetic resonance (^{1}H-NMR) spectrum of CF-3 was identical with that of (−)-epicatechin (**3a**). However, CF-3 exhibited a positive specific optical rotation, and moreover the magnitude [+21.9° (acetone)] was smaller than that [+52.0° (acetone)][3a,7] of (+)-epicatechin (**3b**), suggesting that CF-3 is a mixture of **3a** and **3b**. Based on the additive effect of chiroptical contributions from each enantiomer, the ratio of **3a** and **3b** in CF-3 was calculated to be *ca.* 3:7. Furthermore, HPLC analysis (Fig. 1) using a chiral resolution column (Chiralcel

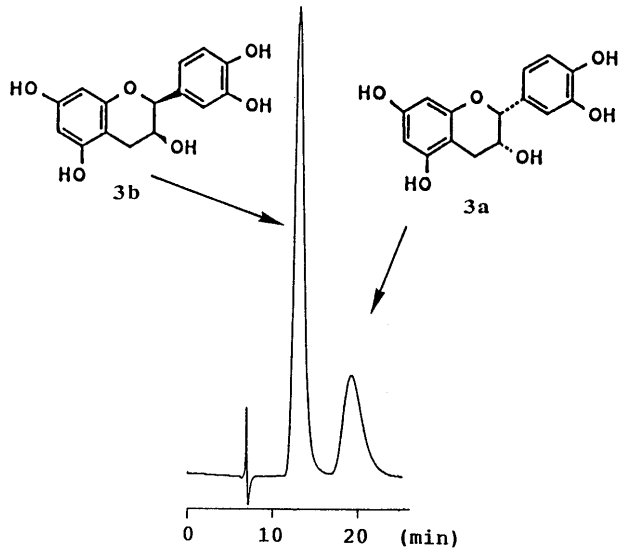
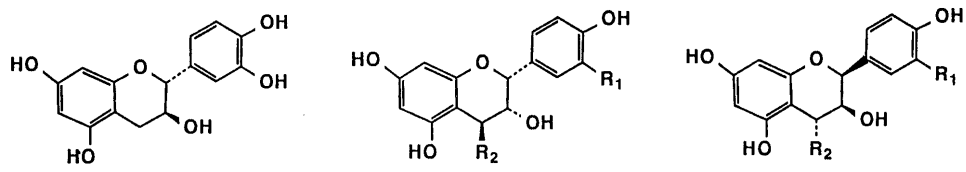


Fig. 1. Chiral Resolution High-Performance Liquid Chromatogram of CF-3

Column, Chiralcel OC; solvent, *n*-hexane–iso-PrOH (0.5% AcOH) (35:65); flow rate, 0.5 ml/min; column temperature, 35 °C; detection, 280 nm.



1

	R_1	R_2
2a	H	H
3a	OH	H
4a	H	$SCH_2C_6H_5$
5a	OH	$SCH_2C_6H_5$

	R_1	R_2
2b	H	H
3b	OH	H
4b	H	$SCH_2C_6H_5$
5b	OH	$SCH_2C_6H_5$

Chart 1

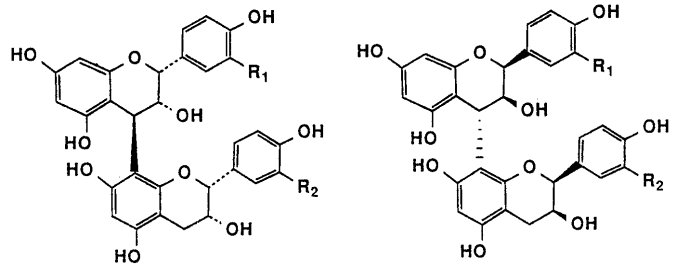
OC) clearly showed two peaks corresponding to **3b** and **3a**. Calibration of each peak area established the ratio of **3a** : **3b** to be 3 : 7.

Since the separation of CF-4 and -5 was very difficult, they were initially considered to be structural isomers. CF-4 showed a ^{1}H-NMR spectrum identical to that of the known procyanidin B-2 (**6a**), but its specific optical rotation [−24.3° (acetone)] differed from that [+32.2° (acetone)][5] of **6a**, suggesting that CF-4 consists mainly of enantiomeric procyanidin B-2 (**6b**).

On the other hand, the ^{1}H-NMR and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of CF-5 were slightly different from that of procyanidin B-2 (**6a**). In the ^{1}H-NMR spectrum, the small coupling patterns of the signals due to flavan H-2, H-2′, and H-4 [δ 5.08 (s), 4.99 (s) and 4.73 (s)] suggested that CF-5 is a dimeric proanthocyanidin consisting of two epicatechin units, whose mode of linkage is similar to that of CF-4. Treatment of CF-5 with benzylmercaptan in the presence of acetic acid[8] afforded a thioether (CF-5a) and a flavan-3-ol (CF-5b), whose ^{1}H-NMR spectra coincided with those of (−)-epicatechin 4β-benzylthioether (**5a**)[5] and (−)-epicatechin (**3a**).[5] Comparison of their specific optical rotations [CF-5a: −6.2° (acetone); CF-5b: +10.9° (acetone)] with those [**5a**: −28.6° (acetone); **3a**: −52.0° (acetone)] of optically pure samples[5] showed that CF-5 is a mixture of proanthocyanidins, the structure being represented by the formulae **10a** and **10b**.

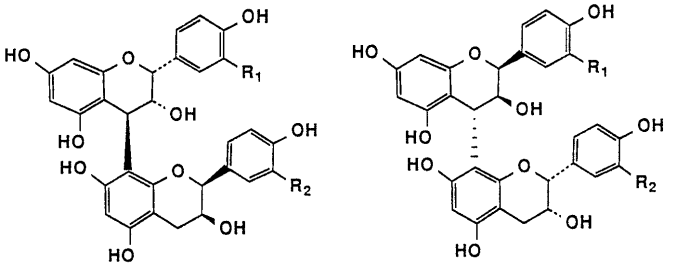
To confirm the proportions of each enantiomer in CF-4 and -5, an attempt was made to analyze CF-4 and -5 directly by chiral resolution HPLC. When a Chiralcel OD column was used, each enantiomer could be separated (Fig. 2), and the proportions of each enantiomer in CF-4 and -5 were roughly calculated from their peak area to be 1 : 9 and 6 : 4 for **6a**, **6b** and **10a**, **10b**, respectively, although the separation of the two peaks under these conditions was unsatisfactory. Furthermore, the flavan-3-ols (CF-4a and -5b) derived from the lower half unit of CF-4 and -5 by thiolytic degradation were subjected to HPLC analysis using a Chiralcel OC column, and it was concluded that the ratios of **6a** : **6b** and **10a** : **10b** are 1 : 9 and 6 : 4, respectively.

CF-6 and -7 and CJ-3 and -4 showed in the negative fast atom bombardment mass spectra (FAB-MS) the same $[M-H]^-$ peak at m/z 545, which is 32 mass units less than that of CF-4 and -5. The ^{1}H-NMR spectrum of CF-6 was identical with that of CJ-3, and also closely correlated with that of procyanidin B-2 (**6a**), except for the observation of



	R_1	R_2
6a	OH	OH
7a	H	H
8a	H	OH
9a	OH	H

	R_1	R_2
6b	OH	OH
7b	H	H
8b	H	OH
9b	OH	H



	R_1	R_2
10a	OH	OH
11a	H	H
12a	H	OH
13a	OH	H

	R_1	R_2
10b	OH	OH
11b	H	H
12b	H	OH
13b	OH	H

Chart 2

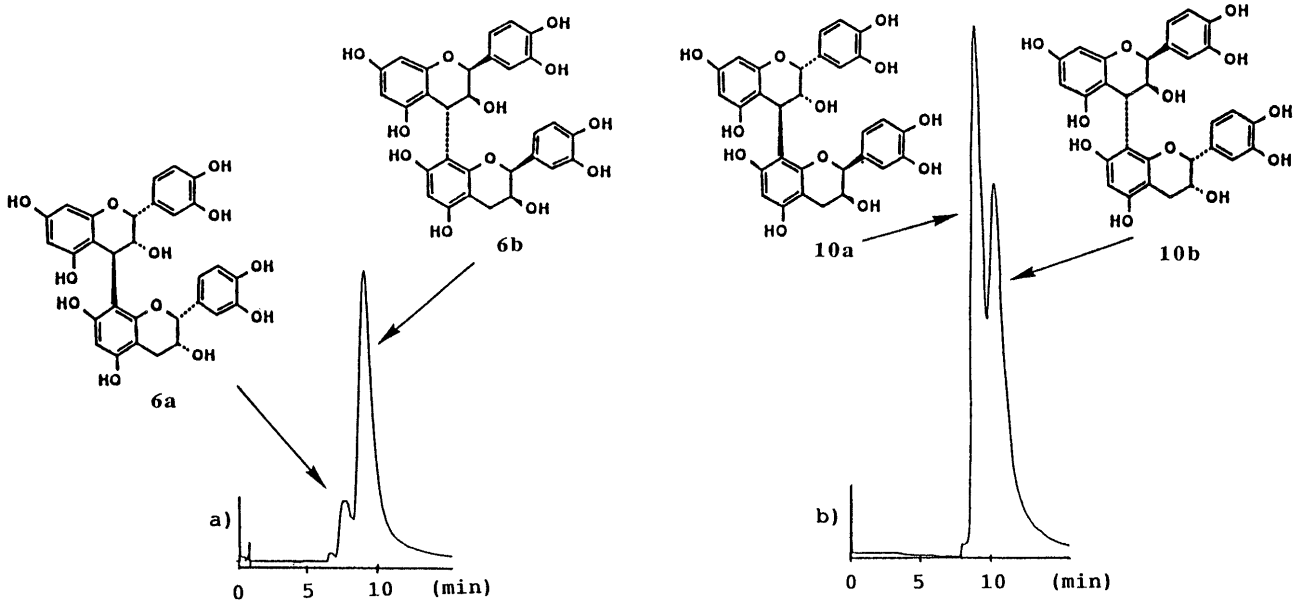


Fig. 2. Chiral Resolution High-Performance Liquid Chromatograms of CF-5(a) and -6(b)

Column, Chiralcel OD; solvent, *n*-hexane–iso-PrOH (0.5% AcOH) (7 : 3); flow rate, 0.7 ml/min; column temperature, 40 °C; detection, 280 nm.

two A_2B_2-type aromatic signals instead of two ABX-type signals, suggesting that they consist of two epiafzelechin units. On the other hand, the specific optical rotations of CF-6 [−25.8° (acetone)] and CJ-3 [+33.2° (acetone)] were quite different, showing the opposite sign. Treatment of CJ-3 with benzylmercaptan in the presence of acetic acid afforded a thioether (CJ-3a) and a flavan-3-ol (CJ-3b). The latter was identified as optically pure (−)-epiafzelechin by comparison of the optical resolution, chiral HPLC retention time and spectral data with those of an authentic sample.[6,9] The thioether was desulfurized with Raney nickel to furnish (−)-epiafzelechin, thus confirming unequivocally the component units. The location and the configuration of the interflavanoid linkage were concluded to be C(4β)–C(8) based on the fact that the chemical shift of the lower flavan H-2 signal[5,10] and the coupling constant of the upper flavan H-4 signal were almost identical with those of procyanidin B-2 (**6a**). Consequently, the structure of CJ-3 was characterized as epiafzelechin-(4β→8)-epiafzelechin (**7a**).

On the other hand, similar thiolytic degradation of CF-6 gave a thioether (CF-6a) and a flavan-3-ol (CF-6b), whose ^{1}H-NMR spectra were identical with those of CJ-3a and -3b, respectively. The sign and the magnitude of the specific optical rotations of CF-6a [+25.6° (acetone)] and -6b [+40.6° (acetone)], as compared with those of CJ-3a [−33.3° (acetone)] and -3b [−52.0° (acetone)], respectively, indicated that CF-6 is partly racemic (a mixture of **7a** and **7b**), and the proportion of **7a** and **7b** was estimated to be 1 : 9 from chiral HPLC analysis of CF-6b.

The ^{1}H-NMR spectra of CF-7 and CJ-4 were identical, and also closely resembled that of CF-5 (**10a** and **10b**), except for the appearance of two A_2B_2-type aromatic signals. Thiolytic degradation of CJ-4 afforded a thioether (CJ-4a) and a flavan-3-ol (CJ-4b), the latter being identified as (−)-epiafzelechin (**2a**). The thioether was converted by desulfurization with Raney nickel into a flavan-3-ol which was found to be identical with (+)-epiafzelechin (**2b**).[3a] Since the chemical shift of the lower H-2 signal and the coupling constant of the upper H-4 signal were analogous to those of 4—8 linked procyanidin B-2,[5] the location and configuration of the interflavanoid linkage were concluded to be C(4α)–C(8). Accordingly, CJ-4 was characterized as *ent*[11]-epiafzelechin-(4α→8)-epiafzelechin (**11b**).

Similar thiolytic degradation of CF-7 afforded a thioether (CF-7a) and a flavan-3-ol (CF-7b), whose ^{1}H-NMR spectra were identical with those of CJ-4a and CJ-4b, respectively. Comparison of their respective specific optical rotations [CF-7a: −15.7° (acetone); CF-7b: +24.6° (acetone)] with those of CJ-4a [+33.0° (acetone)] and CJ-4b [−52.0° (acetone)] indicated that CF-7 is a mixture of **11a** and **11b** in the ratio of 75 : 25, which was consistent with the data obtained by chiral HPLC analysis of CF-7b.

The ^{1}H-NMR spectra of CJ-5 and -6 resembled those of CJ-3 (**7a**) and -4 (**11b**), respectively. However, the appearance of an A_2B_2- and an ABX-type aromatic signals in each case suggested that they consist of epiafzelechin and epicatechin units, in agreement with the negative FAB-MS data [*m/z* 561 $(M-H)^-$]. Thiolytic degradation of CJ-5 afforded (−)-epiafzelechin 4β-benzylthioether (**4a**) and (−)-epicatechin (**3a**), while on similar thiolytic degradation, CJ-6 yielded (+)-epiafzelechin 4α-benzylthioether (**4b**) and (−)-epicatechin (**3a**). Since the chemical shifts of the lower H-2 and the coupling constants of the lower H-4 in CJ-5 and -6 were analogous to those of CJ-3 and -4, the location and the configuration of the interflavanoid linkage in CJ-3 and -4 were concluded to be C(4β)–C(8) and C(4α)–C(8), respectively. Consequently, CJ-5 and -6 were characterized as epiafzelechin-(4β→8)-epicatechin (**8a**) and *ent*-epiafzelechin-(4α→8)-epicatechin (**12b**).

CF-8—CF-11 were initially obtained as a mixture, and separated fully by careful reverse-phase chromatography with monitoring by HPLC. The negative FAB-MS of CF-8—CF-11 showed the same $[M-H]^-$ peak at *m/z* 561, identical with those of CJ-5 and -6. The ^{1}H-NMR spectrum of CF-8 was identical with that of CJ-5 (**8a**), whereas that of CF-9 was the same as that of CJ-6 (**12b**). Comparisons of the respective specific optical rotations of CF-8 and -9 with those of CJ-5 and -6 indicated that CF-8 consists of an enantiomeric mixture of **8a** and **8b**, while CF-9 is a mixture of **12a** and **12b**. Confirmation of the relative proportions of each enantiomer in CF-8 was obtained by chiral HPLC analysis of the flavan-3-ol derived from the lower unit by thiolysis, and the ratio of **8a** and **8b** was found to be 3 : 7. On the other hand, similar HPLC analysis of CF-9 showed that it is practically racemic, although the amount of **12a** is slightly more than that of **12b**.

The ^{1}H-NMR spectra of CF-10 and -11 were similar to those of CJ-5 (**8a**) and -6 (**12b**), respectively, suggesting that CF-10 and -11 also consist of epiafzelechin and epicatechin units. Thiolytic degradation of CF-10 and -11 afforded the corresponding thioethers and flavan-3-ols, which were found to be partly racemic epicatechin 4-benzylthioether and epiafzelechin. Therefore, CF-10 was concluded to be a mixture of procyanidins, the structure being represented by the formulae **9a** and **9b**, and CF-11 to be a mixture of **13a** and **13b**. The measurements of the ratios of each enantiomer in CF-10 and -11 by application of chiral HPLC to the lower flavan-3-ols obtained by thiolytic degradation gave the values of 15 : 85 for **9a** : **9b** and 75 : 25 for **13a** : **13b**.

The trimeric constitution of CJ-7 was confirmed by negative FAB-MS analysis [*m/z* 817 $(M-H)^-$]. The ^{1}H-NMR spectrum showed three flavan H-2 signals whose small coupling constants ($J = ca.$ 0 Hz) suggested that all the favan-3-ol units possess 2,3-*cis* stereochemistry. In addition, the appearance of three A_2B_2-type aromatic signals indicated that CJ-7 consists entirely of epiafzelechin units. Partial thiolytic degradation of CJ-7 furnished *ent*-epiafzelechin-(4α→8)-epiafzelechin (**11b**) and epiafzelechin-(4β→8)-*ent*-epiafzelechin 4′-benzylthioether (CJ-7a), together with (−)-epiafzelechin (**2a**) and optically impure[12] epiafzelechin 4-benzylthioether. The structure of CJ-7a was confirmed by desulfurization with Raney nickel to furnish the enantiomer (**11a**) of naturally occurring **11b**. Therefore, CJ-7 was established as epiafzelechin-(4β→8)-*ent*-epiafzelechin-(4α→8)-epiafzelechin (**14**).

Previously, we reported the isolation and characterization of polyphenolic constituents from the fresh leaves of *Cassia fistula*,[13] in which the structures of two dimeric proanthocyanidins (compounds **5** and **7** in that paper) were represented by formulae **7a** and **9a**. However, the ^{1}H-NMR spectra of compounds **5** and **7** were found to be identical with those of **11b** and **12b**, respectively. Furthermore,

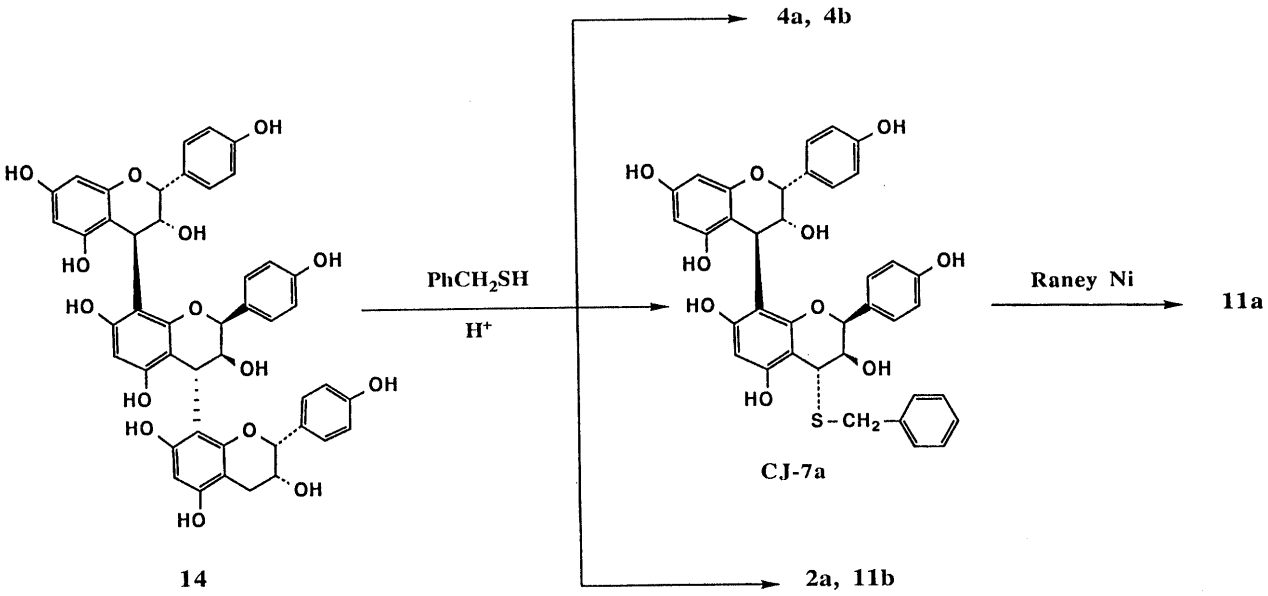


Chart 3

comparisons of their specific optical rotations with those of **11b** and **12b** showed that these compounds are partly racemic. Based on their specific optical rotations, compound **5** was concluded to be a mixture of **11a** and **11b** in a ratio of about 7 : 3, and compound **7** to be a mixture of **12a** and **12b** in a ratio of *ca.* 65 : 35.

As mentioned before, it was formerly believed that only some species of Monocotyledonae and Gymnospermae characteristically produce proanthocyanidins containing a 2*S*-flavan unit. Our present study provides the first example of the occurrence of enantiomeric proanthocyanidins in Dicotyledonous plants.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. FAB-MS were taken with a JEOL JMS DX-300 instrument. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL FX-100 spectrometer, with tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Column chromatography was performed with Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP 20P (75—150 μ, Mitsubishi Chemical Industries, Ltd.), Bondapak C_{18}/Porasil B (35—75 μ, Waters Associates, Inc.) and Kusano packed column ODS (CPO-223L-20-E) (22 mm i.d. × 300 mm, Kusano Kagakukikai Co.). Thin layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merck) with benzene–ethyl formate–formic acid (1 : 7 : 1), and the spots were detected by ultraviolet (UV) illumination and by spraying anisaldehyde–sulfuric acid reagent. HPLC was performed on a Toyo Soda apparatus epuipped with a CCPM solvent delivery system, a UV-8000 spectrometer and a Nucleosil 5C_{18} (M. Nagel) column (4.0 mm i.d. × 250 mm) [mobile phase: CH_3CN–50 mM H_3PO_4 aqueous solution]. For optical resolution of flavan-3-ols and proanthocyanidins, Chiralcel OC and Chiralcel OD (Daicel Chemical Industries, Ltd.) columns (4.6 mm i.d. × 250 mm) [mobile phase: *n*-hexane–isopropanol (0.5% AcOH) (7 : 3, 35 : 65 and 45 : 55); column temp.: 35 °C or 45 °C] were used.

Extraction and Isolation a) The fresh pods of *Cassia fistula* (11.8 kg), collected in Taiwan, were extracted with 80% aqueous acetone at room temperature. After removal of the solvent by evaporation, the extract was subjected to chromatography over Sephadex LH-20 with H_2O containing increasing amounts of MeOH and then with 50% aqueous acetone to give three fractions (fractions I—III). Fraction II was rechromatographed over Sephadex LH-20 with EtOH to furnish two further fractions (fractions II-1 and II-2). Fraction II-1 consisted of flavan-3-ols, which were separated by MCI-gel CHP-20P chromatography [H_2O–MeOH (1 : 0→1 : 1)] to yield CF-1 (80 mg), CF-2 (250 mg) and CF-3 (828 mg). Fraction II-2, consisting of a mixture of dimeric proanthocyanidins, was fractionated by MCI-gel CHP 20P chromatography [H_2O–MeOH (1 : 0→1 : 1)] into three fractions (fractions II-2a—II-2c). Fractions II-2a and II-2b were separately chromatographed over Kusano packed column ODS (18% or 25% MeOH) with monitoring by HPLC to afford CF-4 (347 mg), -5 (578 mg), -8 (132 mg), -9 (90 mg), -10 (25 mg), and -11 (210 mg). Fraction II-2c was chromatographed over Bondapak C_{18}/Porasil B [H_2O–MeOH (1 : 0→1 : 1)] to furnish CF-6 (28 mg) and -7 (42 mg).

b) The dried bark of *C. javanica* (5.1 kg), collected in Taiwan, was chopped into small pieces, and extracted with 80% aqueous acetone at room temperature. After evaporation of the solvent, the extract was chromatographed over Sephadex LH-20 as described above to give three fractions (fractions I—III). Fraction I was rechromatographed over Sephadex LH-20 (EtOH) and MCI-gel CHP 20P [H_2O–MeOH (1 : 0→1 : 1)] to yield CJ-1 (3.0 g) and -2 (280 mg). Repeated chromatography of fraction II over Sephadex LH-20 (acetone), MCI-gel CHP 20P [H_2O–MeOH (1 : 0→1 : 1)] and Kusano packed column ODS (30% MeOH) with monitoring by HPLC afforded CJ-3 (130 mg), -4 (350 mg), -5 (40 mg) and -6 (199 mg). Fraction III was chromatographed over Sephadex LH-20 (EtOH) and Bondapak C_{18}/Porasil B [H_2O–MeOH (1 : 0→1 : 1)] to furnish CJ-7 (130 mg).

General Procedure for Complete Thiolytic Degradation A mixture of each sample (20—150 mg), benzylmercaptan (1—3 ml) and acetic acid (1—4 ml) in EtOH (4—6 ml) was refluxed for 2—3 h with stirring. After cooling, the reaction mixture was directly chromatographed over Sephadex LH-20. Elution with EtOH gave the thioether, and further elution with the same solvent furnished the flavan-3-ol. The thioether and the flavan-3-ol were characterized by physical and spectral comparisons and also by chiral resolution HPLC.

CF-1 [(+)-Catechin (1)] Colorless needles (H_2O), mp 173—175 °C, $[\alpha]_D^{24}$ +14.7° (*c* = 0.69, acetone). ^{1}H-NMR (acetone-d_6): 2.50 (1H, dd, *J* = 8, 16 Hz, H-4), 2.96 (1H, dd, *J* = 6, 16 Hz, H-4), 4.02 (1H, m, H-3), 4.57 (1H, d, *J* = 8 Hz, H-2), 5.90, 6.04 (each 1H, d, *J* = 2 Hz, H-6 and 8), 6.72 (1H, dd, *J* = 2, 8 Hz, H-6′), 6.80 (1H, dd, *J* = 8 Hz, H-5′), 6.90 (1H, d, *J* = 2 Hz, H-2′).

CJ-1 [(−)-Epiafzelechin (2a)] Colorless needles (H_2O), mp 248—250 °C, $[\alpha]_D^{23}$ −51.7° (*c* = 1.1, acetone). ^{1}H-NMR (acetone-d_6 + D_2O): 2.66 (1H, dd, *J* = 4, 17 Hz, H-4), 2.89 (1H, dd, *J* = 4, 17 Hz, H-4), 4.19 (1H, m, H-3), 4.93 (1H, s, H-2), 5.93, 6.04 (each 1H, d, *J* = 2 Hz, H-6 and 8), 6.82, 7.34 (each 2H, d, *J* = 8 Hz, B-ring H).

CF-2 [(+)-Epiafzelechin (2b)] Colorless needles (H_2O), mp 249—251 °C, $[\alpha]_D^{24}$ +52.3° (*c* = 1.01, acetone).

CJ-2 [(−)-Epicatechin (3a)] Colorless needles (H_2O), mp 239—241 °C, $[\alpha]_D^{26}$ −53.6° (*c* = 1.0, acetone). ^{1}H-NMR (acetone-d_6 + D_2O): 2.88 (1H, dd, *J* = 3, 16 Hz, H-4), 3.10 (1H, dd, *J* = 4, 16 Hz, H-4), 4.19 (1H, m, H-3), 4.89 (1H, s, H-2), 5.91, 6.02 (each 1H, d, *J* = 2 Hz, H-6 and 8), 6.75 (1H, d, *J* = 8 Hz, H-5′), 6.90 (1H, dd, *J* = 2, 8 Hz, H-6′), 7.06 (1H, d, *J* = 2 Hz, H-2′).

CF-3 (3a and 3b) Colorless needles (H_2O), mp 227—232 °C, $[\alpha]_D^{24}$

Table I. The Ratio of Enantiomers in CF-1—11 and CJ-1—7

Tentative compd. name	Compd. name	Ratio of enantiomers (+) : (−)
CF-1	Catechin	1 (**1**) : 0
CF-2	Epiafzelechin	0 (**2b**) : 1 (**2a**)
CF-3	Epicatechin	7 (**3b**) : 3 (**3a**)
CF-4	Procyanidin B-2 (**6a**) and its enantiomer (**6b**)	1 (**6a**) : 9 (**6b**)
CF-5	Epicatechin-(4β→8)-*ent*-epicatechin (**10a**) and its enantiomer (**10b**)	6 (**10a**) : 4 (**10b**)
CF-6	Epiafzelechin-(4β→8)-epiafzelechin (**7a**) and its enantiomer (**7b**)	1 (**7a**) : 9 (**7b**)
CF-7	Epiafzelechin-(4β→8)-*ent*-epiafzelechin (**11a**) and its enantiomer (**11b**)	75 (**11a**) : 25 (**11b**)
CF-8	Epiafzelechin-(4β→8)-epicatechin (**8a**) and its enantiomer (**8b**)	3 (**8a**) : 7 (**8b**)
CF-9	Epiafzelechin-(4β→8)-*ent*-epicatechin (**12a**) and its enantiomer (**12b**)	1 (**12a**) ≃ 1 (**12b**)
CF-10	Epicatechin-(4β→8)-epiafzelechin (**9a**) and its enantiomer (**9b**)	15 (**9a**) : 85 (**9b**)
CF-11	Epicatechin-(4β→8)-*ent*-epiafzelechin (**13a**) and its enantiomer (**13b**)	75 (**13a**) : 25 (**13b**)
CJ-1	(−)-Epiafzelechin (**2a**)	0 (**2b**) : 1 (**2a**)
CJ-2	(−)-Epicatechin (**3a**)	0 (**3b**) : 1 (**3a**)
CJ-3	Epiafzelechin-(4β→8)-epiafzelechin (**7a**)	1 (**7a**) : 0 (**7b**)
CJ-4	*ent*-Epiafzelechin-(4α→8)-epiafzelechin (**11b**)	0 (**11a**) : 1 (**11b**)
CJ-5	Epiafzelechin-(4β→8)-epicatechin (**8a**)	1 (**8a**) : 0 (**8b**)
CJ-6	*ent*-Epiafzelechin-(4α→8)-epicatechin (**12b**)	0 (**12a**) : 1 (**12b**)
CJ-7	Epiafzelechin-(4β→8)-*ent*-epiafzelechin-(4α→8)-epiafzelechin (**14**)	1 (**14**) : 0

Table II. ^{13}C-NMR Spectral Data for **7a**, **8a**, **9a(9b)**, **11b**, **12b**, **13b(13a)** and **14**[a]

	7a	**11b**	**8a**	**12b**	**9a, 9b**	**13a, 13b**	**14**
C-2	76.8	77.0	76.7	76.8	76.8	76.6	77.2
C-3	72.7	72.5	72.7	72.4	72.8	72.2	72.3
C-4	36.8	36.7	36.8	36.6	36.8	36.3	36.7
C-2′	79.1	79.4	79.0	79.2	79.2	79.0	77.2
C-3′	66.2	66.7	66.1	66.7	66.2	66.8	66.5
C-4′	28.4	28.3	28.4	28.4	28.6	28.5	36.7
C-2″							79.4
C-3″							66.5
C-4″							28.3

a) Spectra were taken in acetone-d_6 + D_2O at 25.05 MHz.

+21.9° (c=0.74, acetone).

CF-4 (6a and 6b) A tan amorphous powder, $[\alpha]_D^{26}$ −24.3° (c=1.0, acetone). ^{1}H-NMR (acetone-d_6 + D_2O): 2.70, 2.93 (each 1H, d, J=4, 16 Hz, H-4′), 3.98 (1H, s, H-3), 4.32 (1H, m, H-3′), 4.72 (1H, s, H-4), 4.92 (1H, s, H-2′), 5.08 (1H, s, H-2), 6.00—6.08 (3H in total, m, A-ring H), 6.70—7.20 (6H in total, m, B-ring H).

CF-5 (10a and 10b) A tan amorphous powder, $[\alpha]_D^{26}$ +19.1° (c=1.0, acetone). ^{1}H-NMR (acetone-d_6): 2.75 (1H, dd, J=4, 16 Hz, H-4′), 2.96 (1H, dd, J=2, 16 Hz, H-4′), 4.23 (2H, m, H-3 and 3′), 4.73 (1H, s, H-4), 4.79 (1H, s, H-2′), 5.08 (1H, s, H-2), 5.95—6.03 (3H in total, m, A-ring H), 6.69—7.02 (6H in total, m, B-ring H).

CJ-3 (7a) A tan amorphous powder, $[\alpha]_D^{26}$ +33.2° (c=0.9, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{10}\cdot 5/2H_2O$: C, 60.91; H, 5.28. Found: C, 60.65; H, 5.06. Negative FAB-MS m/z: 545 $[M-H]^-$. ^{1}H-NMR (acetone-d_6): 2.56—3.05 (2H, m, H-4′), 4.05 (1H, m, H-3′), 4.30 (1H, s, H-3), 4.75 (1H, s, H-4), 5.01 (1H, s, H-2′), 5.20 (1H, s, H-2), 5.9—6.0 (3H in total, m, A-ring H), 6.76, 6.77, 7.26, 7.39 (each 2H, d, J=8 Hz, B-ring H). ^{13}C-NMR: Table II.

CF-6 (7a and 7b) A tan amorphous powder, $[\alpha]_D^{24}$ −25.8° (c=0.76, acetone).

CJ-4 (11b) A tan amorphous powder, $[\alpha]_D^{26}$ −120.1° (c=1.1, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{10}\cdot 2H_2O$: C, 61.85; H, 5.19. ^{1}H-NMR (acetone-d_6): 2.7—3.1 (2H, m, H-4′), 4.24 (2H, m, H-3 and 3′), 4.74 (1H, s, H-4), 5.03 (1H, s, H-2′), 5.13 (1H, s, H-2), 6.00—6.02 (3H in total, m, A-ring H), 6.75, 6.77 (each 2H, d, J=8 Hz, B-ring H), 7.29 (4H, d, J=8 Hz, B-ring H). ^{13}C-NMR: Table II.

CF-7 (11a and 11b) A tan amorphous powder, $[\alpha]_D^{24}$ +57.1° (c=0.69, acetone).

CJ-5 (8a) A tan amorphous powder, $[\alpha]_D^{19}$ +29.1° (c=1.0, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{11}\cdot 3H_2O$: C, 58.44; H, 5.23. Found: C, 58.57; H, 5.06. Negative FAB-MS m/z: 561 $[M-H]^-$. ^{1}H-NMR (acetone-d_6 + D_2O): 2.64—3.14 (2H, m, H-4′), 3.99 (1H, s, H-3), 4.32 (1H, m, H-3′), 4.71 (1H, s, H-4), 4.96 (1H, s, H-2′), 5.15 (1H, s, H-2), 6.00—6.03 (3H in total, m, A-ring H), 6.7—7.3 (7H in total, m, B-ring H). ^{13}C-NMR: Table II.

CF-8 (8a and 8b) A tan amorphous powder, $[\alpha]_D^{26}$ −11.7° (c=0.66, acetone).

CJ-6 (12b) A tan amorphous powder, $[\alpha]_D^{19}$ −114.6° (c=1.0, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{11}\cdot 3H_2O$: C, 58.44; H, 5.23. Found: C, 58.91; H, 5.02. Negative FAB-MS m/z: 561 $[M-H]^-$. ^{1}H-NMR (acetone-d_6 + D_2O): 2.74—3.21 (2H, m, H-4′), 4.26 (2H, m, H-3 and 3′), 4.70 (1H, s, H-4), 4.99 (1H, s, H-2′), 5.14 (1H, s, H-2), 6.00—6.06 (3H in total, m, A-ring H), 6.6—7.3 (7H in total, m, B-ring H). ^{13}C-NMR: Table II.

CF-9 (12a and 12b) A tan amorphous powder, $[\alpha]_D^{24}$ +4.0° (c=0.98, acetone).

CF-10 (9a and 9b) A tan amorphous powder, $[\alpha]_D^{26}$ −26.0° (c=0.83, acetone). Negative FAB-MS m/z: 561 $[M-H]^-$. ^{1}H-NMR (acetone-d_6): 2.66, 2.95 (each 1H, dd, J=4, 16 Hz, H-4′), 3.98 (1H, s, H-3), 4.28 (1H, m, H-3′), 4.71 (1H, s, H-4), 5.01 (1H, br s, H-2′), 5.12 (1H, s, H-2), 5.90—6.05 (3H in total, m, A-ring H), 6.7—7.4 (7H in total, m, B-ring H). ^{13}C-NMR: Table II.

CF-11 (13a and 13b) A tan amorphous powder, $[\alpha]_D^{26}$ +56.2° (c=0.88, acetone). Negative FAB-MS m/z: 561 $[M-H]^-$. ^{1}H-NMR (acetone-d_6): 2.65—3.05 (2H, m, H-4′), 4.24 (2H, m, H-3 and 3′), 4.74 (1H, s, H-4), 5.05 (1H, s, H-2′), 5.07 (1H, s, H-2), 5.95—6.00 (3H in total, m, A-ring H), 6.8—7.4 (7H in total, m, B-ring H). ^{13}C-NMR: Table II.

CF-7 (14) A tan amorphous powder, $[\alpha]_D^{26}$ +4.9° (c=1.1, acetone). *Anal.* Calcd for $C_{45}H_{38}O_{15}\cdot 4H_2O$: C, 60.67; H, 5.43. Found: C, 61.13; H, 5.07. Negative FAB-MS m/z: 817 $[M-H]^-$. ^{1}H-NMR (acetone-d_6): 2.9—3.1 (2H, m, H-4″), 4.33 (3H, m, H-3, 3′ and 3″), 4.82 (2H, br s, H-4 and 4′), 5.10 (1H, s, H-2″), 5.27 (2H, s, H-2 and 2′), 5.95—6.04 (4H in total, m, A-ring H), 6.77 (6H, d, J=8 Hz, B-ring H), 7.34, 7.41 (6H in total, d, J=8 Hz, B-ring H). ^{13}C-NMR: Table II.

Partial Thiolytic Degradation of CJ-7 A mixture of CJ-7 (200 mg), benzylmercaptan (2 ml) and acetic acid (2 ml) in EtOH (15 ml) was refluxed for 3 h with stirring. After cooling, the reaction mixture was chromatographed over Sephadex LH-20 (EtOH and 80% MeOH) to furnish (−)-epiafzelechin (11 mg), *ent*-epiafzelechin-(4α→8)-epiafzelechin (43 mg), a mixture of 4-benzyl thioethers of (−)-epiafzelechin and (+)-epiafzelechin (7 mg) and a dimeric propelargonidin 4′-benzylthioether (CJ-7a) (20 mg) as a tan amorphous powder, $[\alpha]_D^{24}$ +84.7° (c=0.7, acetone). Negative FAB-MS m/z: 677 $[M-H]^-$. ^{1}H-NMR (acetone-d_6 + D_2O): 4.06 (2H, s, $-SCH_2-$), 4.10—4.18 (3H, m, H-3, 3′ and 4′), 4.70 (1H, s, H-4), 5.11 (1H, s, H-2′), 5.43 (1H, s, H-2), 6.0—6.4 (3H in total, m, A-ring H), 6.78 (4H, d, J=8 Hz, B-ring H), 7.2—7.5 (9H in total, m, B-ring H and aromatic H).

Desulfurization of CJ-7a CJ-7a (20 mg) in 10% AcOH–EtOH (3 ml) was treated with an EtOH slurry of Raney nickel (W-4) at room temperature for 1 h. After removal of the catalyst by filtration, the filtrate was concentrated, and the residue was chromatographed over Sephadex LH-20 [H_2O–MeOH (1 : 0→1 : 4)] to afford CJ-7b (4 mg) as a tan amorphous powder, $[\alpha]_D^{26}$ +112.1° (c=0.6, acetone), whose ^{1}H-NMR spectrum was identical with that of **11b**.

Acknowledgements The authors are grateful to Mr. Y. Tanaka and Miss Y. Soeda for ^{1}H- and ^{13}C-NMR measurements, and Mr. R. Isobe for MS measurements. They are also indebted to the staff of the Central Analysis Room of this university for microanalyses.

References and Notes

1) Part XCII: G. Nonaka, M. Hayashi, T. Tanaka, R. Saijo and I. Nishioka, *Chem. Pharm. Bull.*, **38**, 861 (1990).
2) L. Y. Foo and L. J. Porter, *J. Chem. Soc., Chem. Commun.*, **1982**, 241; *idem*, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 1535; C. J. Ellis, L. Y. Foo and L. J. Porter, *Phytochemistry*, **22**, 483 (1983).
3) *a*) F. D. Monache, F. Ferrari, A. P. Tucci and G. B. M. Bettolo, *Phytochemistry*, **11**, 2333 (1972); *b*) F. D. Monache, F. Ferrari and G.

B. M. Bettolo, *Gazz, Chim. Ital.*, **101**, 387 (1971).
4) *a*) G. Nonaka, S. Morimoto, J. Kinjo, T. Nohara and I. Nishioka, *Chem. Pharm. Bull.*, **35**, 149 (1987); *b*) S. Morimoto, G. Nonaka and I. Nishioka, *ibid.*, **35**, 4217 (1987).
5) G. Nonaka, I. Nishioka, T. Nagasawa and H. Oura, *Chem. Pharm. Bull.*, **29**, 2862 (1981).
6) K. Weinges, G. Goritz and F. Nader, *Justus Liebigs Ann. Chem.*, **715**, 164 (1986).
7) G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **28**, 3145 (1980).
8) R. S. Thompson, D. Jaques, E. Haslam and R. J. Tanner, *J. Chem. Soc., Perkin Trans. 1.*, **1972**, 1387.
9) F. Hashimoto, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **35**, 611 (1987).
10) G. Nonaka, F.-L. Hsu and I. Nishioka, *J. Chem. Soc., Chem. Commun.*, **1981**, 781.
11) According to the proposed nomenclature of proanthocyanidins, naturally uncommon enantiomeric flavan-3-ols are expressed by the prefix *ent-*; R. W. Hemingway, L. Y. Foo and L. J. Porter, *J. Chem. Soc., Perkin Trans. 1.*, **1977**, 1628.
12) Because of the difference in the speed of cleavage reaction of the two interflavanoid linkages, epiafzelechin 4-benzylthioether formed from the upper and middle units are partly racemic.
13) S. Morimoto, G. Nonaka, R.-F. Chen and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 39 (1988).