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Tannins and Related Compounds. LX.[1] Isolation and Characterization of Proanthocyanidins with a Doubly-Linked Unit from *Vaccinium vitis-idaea* L.

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New proanthocyanidin trimers (**14** and **15**) and tetramers (**16** and **17**) have been isolated from the whole body of *Vaccinium vitis-idaea* L. (Ericaceae). The structures of these proanthocyanidins were established by thiolytic degradation and by analyses of the proton and carbon-13 nuclear magnetic resonance spectra. In addition, the presence of (−)-epicatechin (**1**), (+)-catechin (**2**), (−)-epigallocatechin (**3**), (+)-gallocatechin (**4**), procyanidins B-1 (**5**), B-3 (**6**), A-1 (**8**) and A-2 (**9**), and cinnamtannins B_1 (**10**), D_1 (**11**), B_2 (**12**) and D_2 (**13**) in this plant was demonstrated.

Keywords——*Vaccinium vitis-idaea*; Ericaceae; doubly-bonded proanthocyanidin; procyanidin; condensed tannin; flavan-3-ol; thiolytic degradation

In contrast to the wide distribution of singly-linked proanthocyanidins, doubly-linked proanthocyanidins are found only in the members of the limited families, for example, Lauraceae,[2-4] Hippocastanaceae,[1,2,5] Ericaceae,[6] Rosaceae,[7] *etc.* Previously, our chemical studies of Lauraceous[3,4] and Hippocastanaceous plants[1] led to the isolation of a series of the doubly-linked proanthocyanidins, cinnamtannins, from *Cinnamomum zeylanicum* and *C. sieboldii*, and aesculitannins from *Aesculus hippocastanum*. On the other hand, Weinges *et al.* investigated the plants of Ericaceae, and found large amounts of proanthocyanidins A-1 and A-2 in *Vaccinium vitis-idaea*.[6] However, there have been no reports on the isolation of higher oligomeric proanthocyanidins from this plant source. We have now re-examined the proanthocyanidins in *Vaccinium vitis-idaea*, and isolated four new proanthocyanidins with doubly-bonded structures, together with the known flavan-3-ols and proanthocyanidins. This paper describes the isolation and structure elucidation of these compounds.

The whole plant of *Vaccinium vitis-idaea* was extracted with 60% aqueous acetone, and the extract was chromatographed over Sephadex LH-20, MCI-gel CHP 20P and Bondapak C_{18}/Porasil B with various solvent systems to yield compounds **1**—**17**. Compounds **1**—**7** were identified as (−)-epicatechin (**1**),[4] (+)-catechin (**2**),[4] (−)-epigallocatechin (**3**),[8] (+)-gallocatechin (**4**),[9] and procyanidins B-1 (**5**),[4] B-3 (**6**)[9] and B-7 (**7**)[4] by comparisons of physical and spectral data with those of authentic samples. Compounds **8**—**13** were shown to possess doubly-linked structures by analyses of the proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra, and their physical and spectral data coincided with those of proanthocyanidins A-1 (**8**)[5] and A-2 (**9**),[3] and cinnamtannins B_1 (**10**),[4] D_1 (**11**),[4] B_2 (**12**)[4] and D_2 (**13**),[4] respectively.

Compounds **14** and **15** were positive to the anisaldehyde-sulfuric acid (orange-red) and ferric chloride (dark green) reagents. The triflavanoid constitution of each compound was shown by analyses of the negative fast atom bombardment mass spectra (FAB-MS) [*m/z*: 863 $(M-H)^-$].

The ^{13}C-NMR spectrum of **14** exhibited signals at δ 76.9 and 84.5 due to flavan C-2

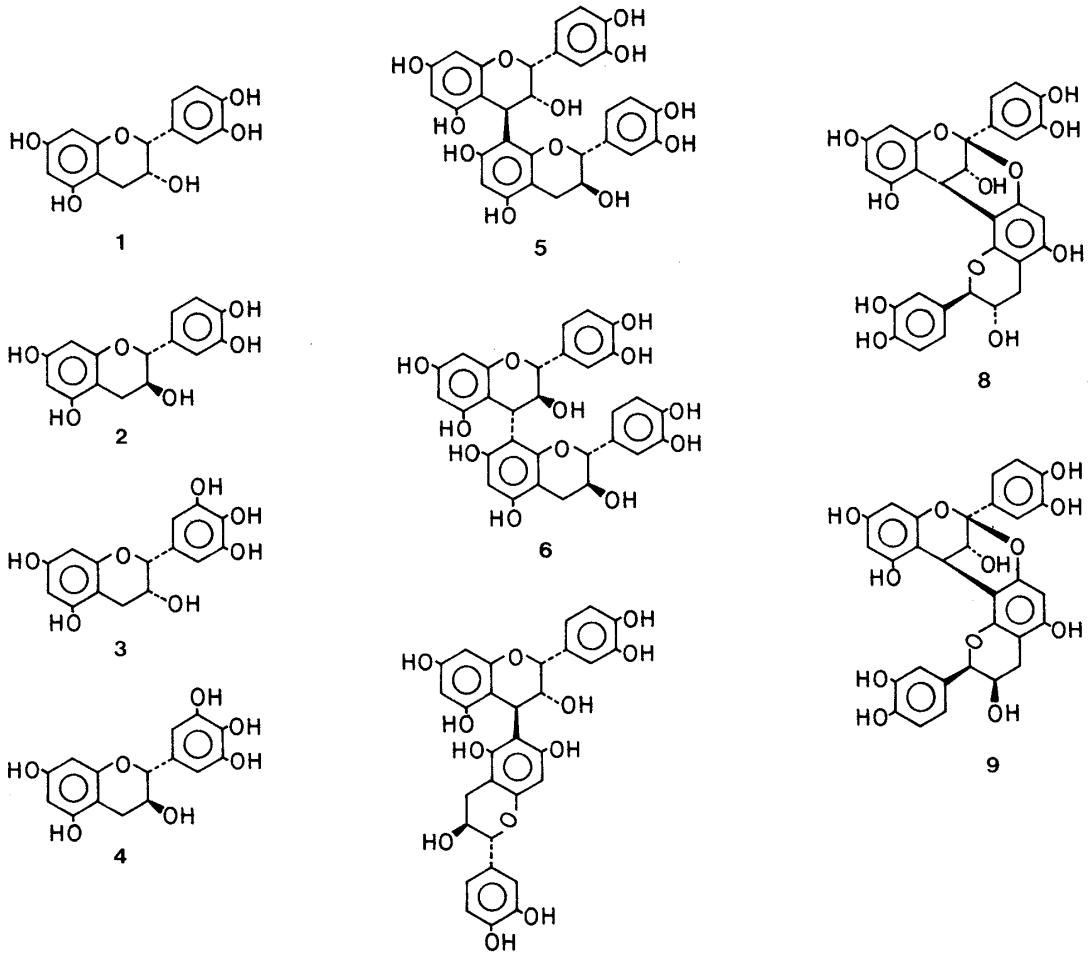
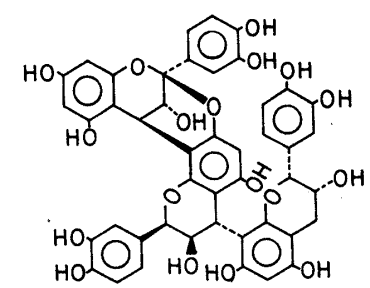


Chart 1

carbons, and the chemical shifts of these signals implied the presence of epicatechin and catechin units in the molecule.[10] In addition, the occurrence of a proanthocyanidin A-type unit was deduced from the chemical shift (δ 104.1) of one of the C-2 resonances,[2] which was consistent with that of a ketal carbon in A-type proanthocyanidins. On the other hand, **15** gave complicated ^{13}C- and ^{1}H-NMR spectra, owing to the existence of several conformers.[11] However, ^{13}C-NMR signals arising from the major conformer could be assigned, showing a close similarity to those in **14**. The spectrum showed a ketal carbon signal at δ 103.6, along with C-2 signals arising from epicatechin (δ 76.9) and catechin (δ 85.2) moieties, thus suggesting that **15** contains the same component units as **14**.

In order to establish the structures of component units in **14** and **15**, cleavage of the interflavanoid linkages was attempted. Treatment of **14** and **15** with benzylmercaptan in the presence of acid[11] afforded the same degradation products, namely (−)-epicatechin 4-benzylthioether (**18**) (formed from the upper unit) and proanthocyanidin A-1 (**8**) (from the lower two units), which were identified by comparisons of the physical and spectral data.

The positions of the interflavanoid linkages between the component units were determined as follows. As described above, **14** gave a first-order ^{13}C-NMR spectrum, but the ^{13}C-NMR spectrum of **15** was complicated by conformational isomerism. This conformational isomerism was considered to be probably caused by steric hindrance to free rotation about the interflavanoid linkages.[3] Examination of a Dreiding model revealed that in the case of the 4,6-linked isomer, the B- and B″-rings were close together and this may be responsible for



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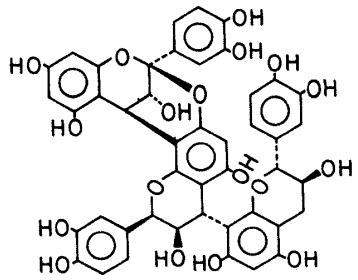

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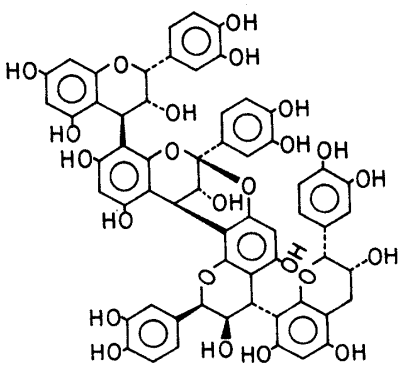

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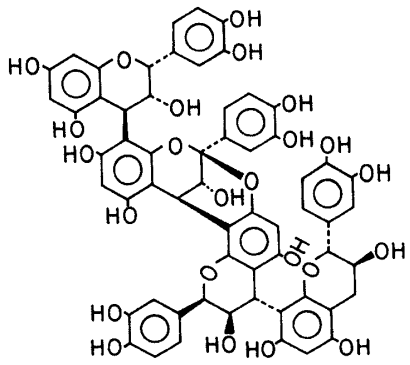

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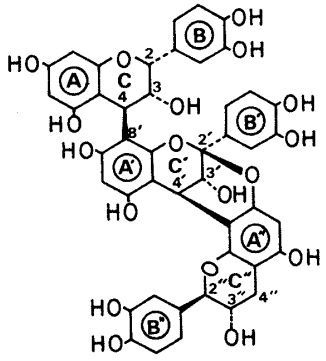
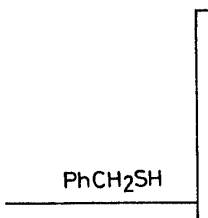
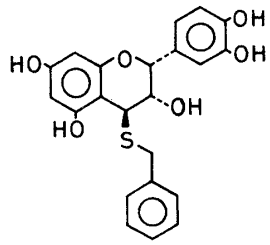
Chart 2



14





18

8

Chart 3

steric hindrance, while in the case of the 4,8-linked isomer such steric hindrance was not observed. These findings indicated that the component units of **14** and **15** are linked through 4,8- and 4,6-interflavanoid linkages, respectively. The configuration of the interflavanoid linkage was determined to be β on the basis of the chemical shift (δ 76.6) of the C-2 signal in each case.[10)]

Accordingly, the structures of **14** and **15** were established as epicatechin-(4β→8)-epicatechin-(4β→8, 2β→*O*→7)-catechin and epicatechin-(4β→6)-epicatechin-(4β→8, 2β→*O*→7)-catechin.

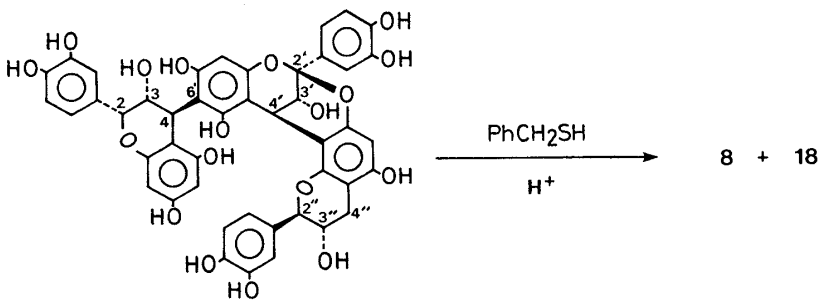


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Chart 4

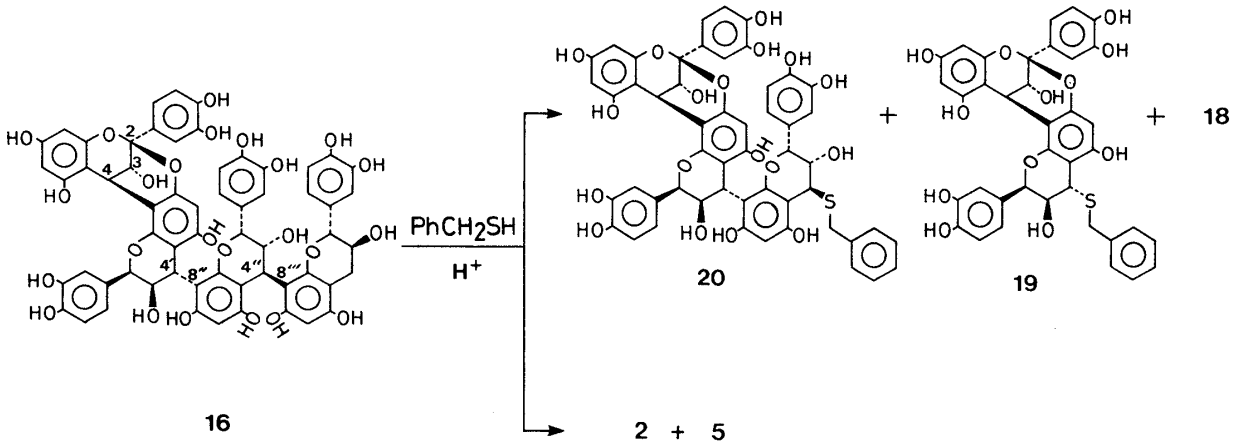
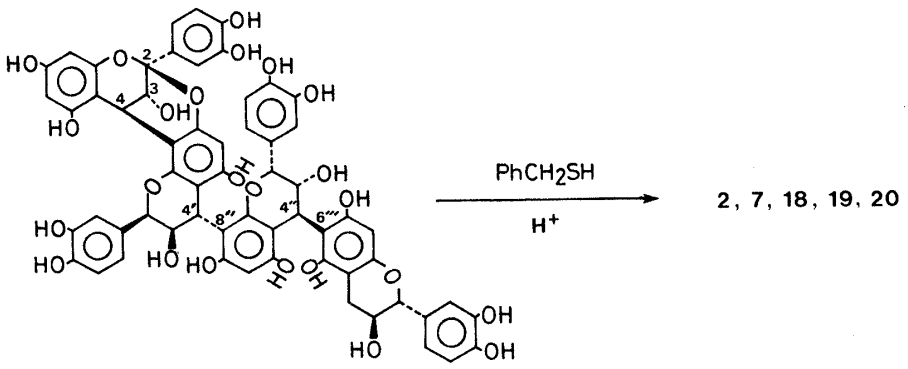


Chart 5

Compound **16** showed, in the negative FAB-MS, the $(M-H)^-$ ion peak at *m/z* 1151, consistent with a tetrameric nature. The ^{13}C-NMR spectrum of **16** showed three flavan C-2 signals at δ 81.5, 78.3 and 76.6. The chemical shift of the former signal was consistent with the presence of a catechin unit, while the latter two signals were attributed to the C-2 carbons in the epicatechin moieties. Furthermore, the presence of a proanthocyanidin A-type unit was revealed by the ketal carbon resonance at δ 104.6. Confirmation of the structures of the component units was effected by complete thiolytic degradation to afford proanthocyanidin A-2 4′-benzylthioether (**19**), (−)-epicatechin 4-benzylthioether (**18**) and (+)-catechin (**2**).

The configurations and the positions of the interflavanoid linkages were determined as follows. On partial thiolytic degradation, **16** gave procyanidin B-1 (**5**) and a thioether (**20**), together with the above degradation products. The thioether was found to be identical with cinnamtannin B_1 4″-benzylthioether (**20**), which was previously obtained by similar thiolytic degradation of aesculitannin E.[1] The formation of **20** and **5** established the structure of **16** to be epicatechin-(4β→8, 2β→*O*→7)-epicatechin-(4α→8)-epicatechin-(4β→8)-catechin.

Compound **17** was shown to consist of the same component units as **16** by analyses of the ^{13}C-NMR spectrum and negative FAB-MS [*m/z*: 1151 $(M-H)^-$] and by complete thiolytic degradation which gave proanthocyanidin A-2 4′-benzylthioether (**19**), (−)-epicatechin 4-benzylthioether (**18**) and (+)-catechin (**2**). The locations and the modes of the interflavanoid linkages were confirmed by partial thiolytic degradation. On similar treatment, **17** furnished procyanidin B-7 (**7**) and cinnamtannin B_1 4″-benzylthioether (**20**), together with the above degradation products. Accordingly, **17** was characterized as epicatechin-(4β→8, 2β→*O*→7)-epicatechin-(4α→8)-epicatechin-(4β→6)-catechin.



17

Chart 6

In conclusion, *Vaccinium vitis-idaea* was found to contain a variety of oligomeric proanthocyanidins containing a doubly-linked A-type unit, in addition to singly-linked procyanidins. Compounds **14** and **15** represent the first examples of condensed tannins possessing a proanthocyanidin A-1 unit.

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. ^{1}H- and ^{13}C-NMR spectra were obtained with JEOL PS-100 and FX-100 instruments. FAB-MS were recorded on a JEOL JMS DX-300 machine. Column chromatography was carried out with Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemical Co., Ltd.), Bondapak C_{18}/Porasil B (37—75 μm, Waters Associates, Inc.) and MCI-gel CHP 20P (75—150 μm, Mitsubishi Chemical Industries, Ltd.). Thin-layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merck) with benzene–ethyl formate–formic acid (2 : 7 : 1 or 1 : 7 : 1), and spots were detected by the use of anisaldehyde-sulfuric acid and ferric chloride reagent sprays.

Extraction and Isolation——The dried whole plants of *Vaccinium vitis-idaea* (12.5 kg) were extracted with 60% aqueous acetone at room temperature. The acetone was evaporated off under reduced pressure, and the resulting precipitates, which consisted mainly of chlorophylls and waxes, were removed by filtration. The filtrate was concentrated and subjected to Sephadex LH-20 chromatography. Elution with H_2O containing increasing amounts of MeOH gave three fractions; fr. 1 (18.5 g), fr. 2 (98.2 g) and fr. 3 (48.5 g). Fr. 1 was rechromatographed over MCI-gel CHP 20P with 30% aqueous MeOH, followed by crystallization, to give (−)-epicatechin (**1**) (2.5 g), (+)-catechin (**2**) (10.5 g), (−)-epigallocatechin (**3**) (32 mg) and (+)-gallocatechin (**4**) (21 mg). Fr. 2 was further divided by Sephadex LH-20 chromatography (EtOH) into three fractions; fr. 2a (25 g), fr. 2b (28 g) and fr. 2c (19 g). Repeated chromatography of fr. 2a on Sephadex LH-20 (60% aqueous MeOH) and MCI-gel CHP 20P (30% aqueous MeOH) yielded procyanidins B-1 (**5**) (1.1 g), B-3 (**6**) (12.5 g) and B-7 (300 mg). Fr. 2b was subjected to Sephadex LH-20 chromatography (80% aqueous MeOH) to give proanthocyanidins A-1 (**8**) (9.5 g) and A-2 (**9**) (8.1 g). Fr. 2c was repeatedly chromatographed over Sephadex LH-20 (60% aqueous MeOH), MCI-gel CHP 20P (30% aqueous MeOH) and Bondapak C_{18}/Porasil B (25% aqueous MeOH) to afford cinnamtannins B_1 (**10**) (1.2 g) and D_1 (**11**) (3.2 g), and compounds **14** (2.9 g) and **15** (1.8 g). Fr. 3, after chromatography on Sephadex LH-20 (EtOH) and MCI-gel CHP 20P (30% aqueous MeOH), furnished cinnamtannins B_2 (**12**) (1.2 g) and D_2 (**13**) (3.5 g), and compounds **16** (1.3 g) and **17** (4.6 g).

Compound 14——An off-white amorphous powder, $[\alpha]_D^{18}$ +38.2° (c=1.0, acetone). *Anal.* Calcd for $C_{45}H_{36}O_{18}\cdot 2H_2O$: C, 60.00; H, 4.48. Found: C, 60.32; H, 4.21. FAB-MS m/z: 863 $(M-H)^-$. ^{1}H-NMR (acetone-d_6) δ: 2.40—3.20 (2H, m, H-4″), 4.28 (2H, s, H-3′, 4′), 4.64 (1H, d, J=8 Hz, H-2″), 4.70 (1H, s, H-4), 5.00 (1H, br s, H-2), 5.80—6.20 (4H in total, m, A-ring H), 6.40—7.40 (9H in total, m, B-ring H). ^{13}C-NMR (acetone-d_6) δ: 36.9 (C-4), 66.9 (C-3′), 67.4 (C-3″), 72.7 (C-3), 76.9 (C-2), 84.5 (C-2″), 104.1 (C-2′).

Thiolytic Degradation of 14——A mixture of **14** (200 mg), benzylmercaptan (2 ml) and acetic acid (2 ml) in EtOH (10 ml) was refluxed for 13 h with stirring. The reaction mixture was concentrated under reduced pressure to afford an oily residue, which was chromatographed over Sephadex LH-20. Elution with EtOH afforded (−)-epicatechin 4-benzylthioether (**18**) (28 mg) and proanthocyanidin A-1 (**8**) (54 mg).

Compound 15——An off-white amorphous powder, $[\alpha]_D^{20}$ +59.5° (c=1.0, acetone). *Anal.* Calcd for $C_{45}H_{36}O_{18}\cdot 2H_2O$: C, 60.00; H, 4.48. Found: C, 59.59; H, 4.39. FAB-MS m/z: 863 $(M-H)^-$. ^{13}C-NMR (acetone-d_6)

δ: 36.8 (C-4), 67.3 (C-3′), 68.0 (C-3″), 72.7 (C-3), 76.9 (C-2), 85.2 (C-2″), 103.6 (C-2′).

Thiolytic Degradation of 15——A mixture of **15** (150 mg), benzylmercaptan (1.5 ml) and acetic acid (2 ml) in EtOH (10 ml) was heated under reflux for 13 h with stirring. The reaction mixture was treated as described for **14** to give (−)-epicatechin 4-benzylthioether (**18**) (12 mg) and proanthocyanidin A-1 (**8**) (24 mg).

Compound 16——An off-white amorphous powder, $[\alpha]_D^{20}$ +104.3° (c=1.0, MeOH). *Anal.* Calcd for $C_{60}H_{48}O_{24}\cdot 3H_2O$: C, 59.70; H, 4.51. Found: C, 59.50; H, 4.91. FAB-MS m/z: 1151 $(M-H)^-$. ^{13}C-NMR (acetone-d_6) δ: 37.0, 38.3 (C-4′, 4″), 66.5, 67.6 (C-3, 3‴), 71.5, 72.1 (C-3′, 3″), 76.6, 78.3, (C-2′, 2″), 81.5 (C-2‴), 104.6 (C-2).

Complete Thiolytic Degradation of 16——A mixture of **16** (100 mg), benzylmercaptan (1.5 ml) and acetic acid (2 ml) in EtOH (10 ml) was refluxed for 10 h with stirring. Work-up as before gave proanthocyanidin A-2 4′-benzylthioether (**19**) (28 mg), (−)-epicatechin 4-benzylthioether (**18**) (9 mg) and (+)-catechin (**2**) (13 mg).

Partial Thiolytic Degradation of 16——A mixture of **16** (750 mg), benzylmercaptan (4 ml) and acetic acid (2 ml) in EtOH (20 ml) was refluxed for 6 h with stirring. The products were separated by repeated chromatography over Sephadex LH-20 with EtOH and 80% aqueous MeOH to give cinnamtannin B_1 4″-benzylthioether (**20**) (250 mg), procyanidin B-1 (**5**) (4.5 mg), proanthocyanidin A-2 4′-benzylthioether (**19**) (56 mg), (−)-epicatechin 4-benzylthioether (**18**) (12 mg) and (+)-catechin (**2**) (19 mg).

Compound 17——A off-white amorphous powder, $[\alpha]_D^{20}$ +74.3° (c=1.0, MeOH). *Anal.* Calcd for $C_{60}H_{48}O_{24}\cdot 3H_2O$: C, 59.70; H, 4.51. Found: C, 59.41; H, 4.67. FAB-MS m/z: 1151 $(M-H)^-$. ^{13}C-NMR (acetone-d_6) δ: 37.4, 38.8 (C-4′, 4″), 66.4 (C-3), 68.0 (C-3‴), 71.8, 72.0 (C-3′, 3″), 77.2, 78.3 (C-2′, 2″), 81.6 (C-2‴), 104.5 (C-2).

Complete Thiolytic Degradation of 17——A mixture of **17** (100 mg), benzylmercaptan (1.5 ml) and acetic acid (2 ml) in EtOH (10 ml) was refluxed for 10 h with stirring. The reaction mixture was treated as before to afford proanthocyanidin A-2 4′-benzylthioether (**19**) (25 mg), (−)-epicatechin 4-benzylthioether (**18**) (12 mg) and (+)-catechin (**2**) (11 mg).

Partial Thiolytic Degradation of 17——A mixture of **17** (500 mg), benzylmercaptan (4 ml) and acetic acid (2 ml) in EtOH (20 ml) was refluxed for 6 h with stirring. The reaction mixture was worked up in the same way as described for **16** to give cinnamtannin B_1 4″-benzylthioether (**20**) (188 mg), procyanidin B-7 (**7**) (10 mg), proanthocyanidin A-2 4′-benzylthioether (**19**) (46 mg), (−)-epicatechin 4-benzylthioether (**18**) (6 mg) and (+)-catechin (**2**) (10 mg).

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References and Notes

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