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4-CARBOXYMETHYL FLAVAN-3-OLS AND PROCYANIDINS FROM *DAVALLIA DIVARICATA**

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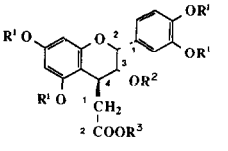
Key Word Index—*Davallia divaricata*, Davalliaceae, fern, 4-carboxy-methylflavan-3-ols, carboxymethylprocyanidins; procyanidins, condensed tannin

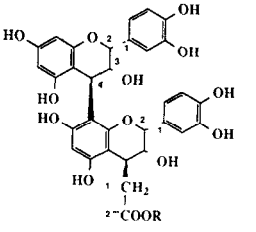
Abstract—A series of flavan-3-ols and procyanidins, possessing a carboxymethyl group, and a procyanidin tetramer have been isolated from the fern, *Davallia divaricata* The structures were characterized on the basis of chemical and spectral evidence.

INTRODUCTION

In the course of our chemical studies on condensed tannins in ferns, we previously demonstrated the presence of flavan-3-ol and proanthocyanidin allosides, along with procyanidins B-1 and B-2 and a trimeric procyanidin, in the rhizomes of *Davallia divaricata* Blume (Davalliaceae) [2]. Further chemical examination of this plant has resulted in the isolation of a series of flavan-3-ols and procyanidins possessing a carboxymethyl group at the C-4 position, together with a new tetrameric procyanidin. This paper describes the isolation and characterization of these compounds



	R^1	R^2	R^3
1	H	H	H
1a	Me	H	Me
1b	Me	C-C_6H_5 (O)	Me
2	H	H	K,Na
3	H	H	Me



4 R=H
5 R=K, Na

RESULTS AND DISCUSSION

The aqueous acetone extract of the fresh rhizomes of *D. divaricata was* repeatedly chromatographed over Sephadex LH-20 and various reverse-phase gels to afford compounds **1**–**6**. Compound **1** (negative FABMS *m/z*: 347[M−H]$^-$) showed an orange colour characteristic of flavan-3-ol derivatives with the anisaldehyde–sulphuric acid reagent The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of epicatechin, and the small coupling constants (each *s*) of H-2 and H-3 signals clearly indicated the 2,3-*cis* configuration of the flavan moiety. In addition, the observation of a methylene (δ39.1) and a carboxylic acid (δ174 4) carbon signals, as well as the IR absorption band at 1710 cm^{-1}, indicated the presence of a carboxymethyl group. In the ^{1}H NMR spectrum, the carboxymethyl signals [δ2.44 (1H, *dd*, J=11, 16 Hz) and 3.05 (1H, *dd*, J=4, 16 Hz)] were found to be coupled with a methine signal [δ3 45 (*dd*, J=4, 11 Hz)], which was assignable to flavan H-4 on the basis of the ^{1}H–^{1}H COSY spectral examination, indicating that the carboxymethyl group is located at the flavan C-4 position. This was further supported by EIMS of the pentamethylate (**1a**), which showed, together with a peak due to [M]$^+$ at *m/z* 418, prominent peaks at *m/z* 239 and 180 formed by a retro-Diels–Alder-type fission of the flavan C-ring (Scheme 1).

The absolute stereochemistry of **1** was determined as follows The ^{1}H–^{1}H NOESY spectrum of **1** showed a cross peak between H-2 signal (δ4.93, s) and one (δ2.44) of the methylene proton signals, indicating that the carboxymethyl group at the C-4 and H-2 are mutually located on the same side. Furthermore, the CD curve of the tetramethyl-3-*O*-benzoate (**1b**) of **1** was in good accord with that of tetramethyl-(−)-epicatechin 3-*O*-benzoate (**7**) Thus, the absolute configurations at the C-2 and C-3 in **1** are same as those of (−)-epicatechin On the basis of these observations, **1** was characterized as 4β-carboxymethyl-(−)-epicatechin

Compound **2** gave ^{1}H and ^{13}C NMR spectra similar to those of **1**, and yielded the same methylate (**1a**) on methylation with dimethyl sulphate and potassium carbonate in dry acetone. However, in the ^{1}H NMR spectrum of **2**, the coupling patterns of H-4 and methylene signals were slightly different from those found in **1**. Furthermore, the IR spectrum of **2** showed no free carboxylic acid absorption around 1700 cm^{-1}, but instead exhibited absorption bands due to a carboxylate (1550 and 1400 cm^{-1}), suggesting that **2** is a salt of **1**. This was further confirmed by treatment of **2** with dilute

*Part 89 in the series 'Tannins and Related Compounds' For. Part 88 see ref. [1]

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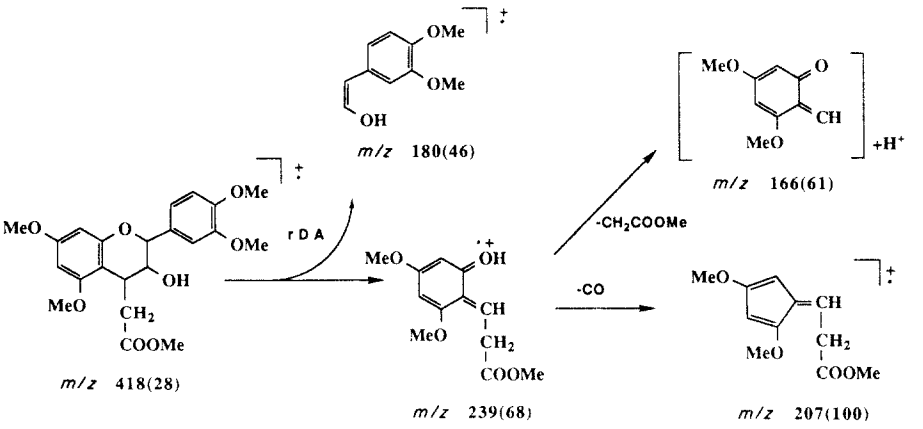


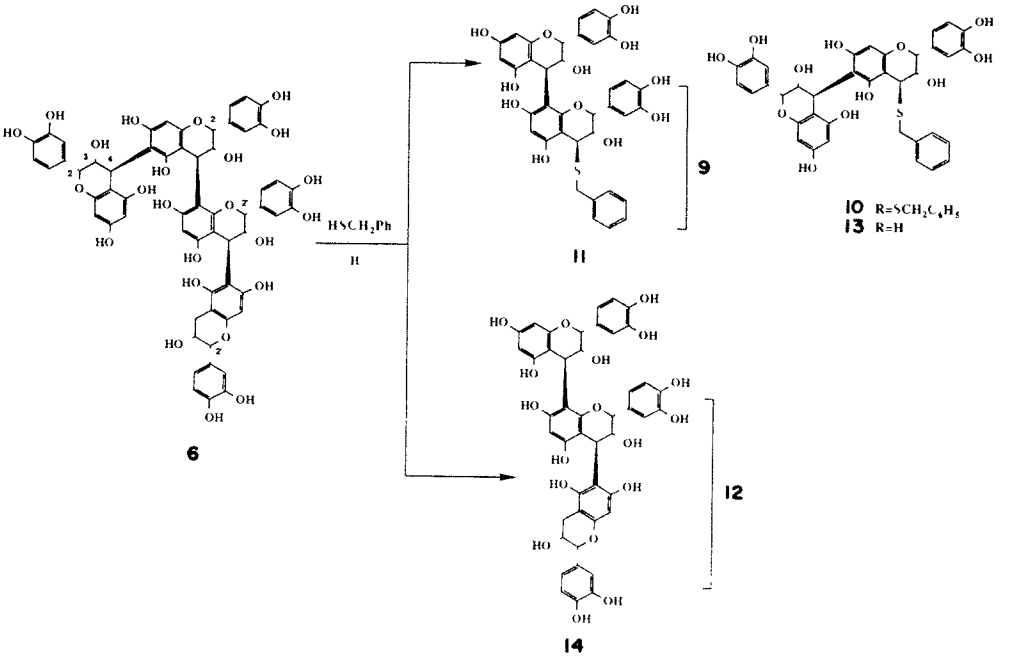
Scheme 1 Fragment ions (relative intensities in parentheses) in EIMS of **1a**

hydrochloric acid to furnish **1** The positive FABMS of **2** showed peaks at *m/z* 387 and 371, which corresponded to the molecular masses of **1** plus K and Na, respectively Moreover, energy dispersing X-ray analysis showed the presence of potassium and sodium ions in **2** From these observations, **2** was concluded to be a mixture of potassium and sodium salts of **1**

The ^{1}H NMR spectrum of **3** closely resembled that of **1**, except for the appearance of a carbomethoxyl signal at δ3 68 The negative FABMS (*m/z* 351 $[M-H]^-$) was consistent with the methyl ester of **1** Final structural confirmation was obtained by treatment of **1** with *p*-toluene-sulphonic acid in methanol, which afforded **3**

The ^{1}H and ^{13}C NMR spectra of **4** were closely correlated with that of procyanidin B-2(**8**), but differed in the observation of additional signals at δ40 0 and 175 7 assignable to methylene and carboxyl carbons, respectively These spectral data, combined with FABMS analysis ($[M-H]^-$ *m/z* 577], suggested **4** to be a carboxymethylprocyanidin B-2. Acid-catalysed degradation of **4** in the presence of benzylmercaptan [3] afforded (−)-epicatechin 4β-benzylthioether (**9**) (formed from the upper half) and **1** (from the lower half) The location and the configuration of the interflavonoid linkage were concluded to be C(4β)−C(8) based on the fact that the chemical shift of the lower H-2 signal and the coupling constant of the upper H-4 signal were almost identical with those observed in **8** From these chemical and spectral data, **4** was characterized as epicatechin-(4β→8)-4β-carboxymethylepicatechin

The ^{1}H and ^{13}C NMR spectra of **5** were quite similar to those of **4**, and thiolytic degradation afforded the same



products (**9** and **1**). The IR spectrum of **5** however showed no carboxylic acid absorption. Furthermore, the positive FABMS showed peaks at *m/z* 675 and 659 corresponding to the potassium and sodium salts of **4**, respectively The presence of the potassium and sodium metals in **5** was similarly confirmed by energy dispersing X-ray analysis. From these observations, **5** was concluded to be a mixture of potassium and sodium carboxylates of **4**.

The tetrameric constitution of **6** was confirmed by negative FABMS [(M−H)$^-$: *m/z* 1153] The ^{13}C NMR spectrum showed signals at δ79.2(1C) and 76.7(3C) due to flavan C-2 carbons whose chemical shifts suggested that **6** consists entirely of epicatechin units [7]. Partial thiolytic degradation of **6** furnished (−)-epicatechin 4β-benzylthioether (**9**), procyanidin B-5 4′β-benzylthioether (**10**) [4], procyanidin B-2 4′β-benzylthioether (**11**) [5], (−)-epicatechin (**12**), procyanidin B-5 (**13**), and epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin (**14**) [6]. Among these products, **14** was considered to be formed from the lower three units, thus establishing unequivocally the configurations and locations of the interflavonoid linkages in the lower three units Furthermore, the formation of **10** indicated that the upper two units are linked through C(4β)-C(6) linkage. On the basis of these observations, the structure of **6** was established as epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin.

Compounds **2**, **5** and **6** have also been isolated from the rhizomes of the allied species, *Davallia griffithiana*, together with (−)-epicatechin 5-*O*-β-D-glucopyranoside, (+)-catechin 5-*O*-β-D-glucopyranoside, and procyanidins B-1 and B-2 However, flavan-3-ol and proanthocyanidin allosides, which had been found to be major compounds in *D. divaricata*, could not be obtained from *D. griffithiana* [except for (−)-epicatechin 3-*O*-β-D-allopyranoside].

EXPERIMENTAL

Details of the instruments and chromatographic conditions used in this work were essentially the same as described in the previous paper [8] Plant materials were collected at San-Chi-Mon near Ping-tung City, Taiwan, and voucher specimens are deposited at the Herbarium, Tajen Pharmaceutical College

Extraction and isolation The fresh rhizomes of *Davallia divaricata* (9 3 kg) were chopped into small pieces and extracted ×3 with 80% aq. Me_2CO at room temp The extract, after removal of the solvent by evapn, was subjected to chromatography over Sephadex LH-20 using H_2O with increasing amounts of MeOH (1·0→0 1) to give four fractions, frs 1 (34 5 g), 2 (6 2 g), 3 (15 8 g) and 4 (13 0 g). Fraction 3 was chromatographed over Sephadex LH-20 (EtOH) to give five fractions (3-1–3-5) Repeated CC of fraction 3-2 over MCI-gel CHP 20P[H_2O–MeOH(1 0→3 2)], LC-sorb DPH[H_2O–MeOH(1.0→3 2)] and Sephadex LH-20 (Me_2CO) afforded compounds **1** (525 mg), **2** (88 mg) and **3** (61 mg) Fraction 3–5 was further chromatographed over prep-PAK 500/C_{18} [H_2O–MeOH(1 0→1 1)] and MCI-gel CHP 20P[H_2O–MeOH(1 0→1 1)] to furnish compounds **4** (25 mg) and **5** (90 mg) Repeated CC of fraction 4 over Sephadex LH-20 (EtOH) and Bondapak C_{18}/Porasil B[H_2O–MeOH (1 0→1 1)] yielded compound **6** (301 mg)

4β-Carboxymethyl-(−)-epicatechin (**1**) An amorphous powder, $[\alpha]_D^{24}$ −46 4° (Me_2CO, *c* 0 7) Negative FABMS *m/z*· 347 [M−H]$^-$ IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 3000 (COOH), 1710 (C=O), 1610, 1520 (C=C) ^{1}H NMR (Me_2CO-d_6) δ2 44 (1H, *dd*, *J* = 11, 16 Hz, H-1″), 3 05 (1H, *dd*, *J* = 4, 16 Hz, H-1″), 3 45 (1H, *dd*, *J* = 4, 11 Hz, H-4), 4 02 (1H, *s*, H-3), 4 93 (1H, *s*, H-2), 5 93, 6.05 (each 1H, *d*, *J* = 2 Hz, H-6, 8), 6 88 (1H, *dd*, *J* = 2, 8 Hz, H-6′), 6 79 (1H, *d*, *J* = 8 Hz, H-5′), 7.08 (1H, *d*, *J* = 2 Hz, H-2′) ^{13}C NMR (Me_2CO-d_6) δ35 9 (C-4), 39 1 (C-1″), 70.2 (C-3), 75.2 (C-2), 95 8, 96 5 (C-6, 8), 102 6 (C-4a), 115.2 (C-2′), 115.5 (C-5′), 119 2 (C-6′), 131 9 (C-1′), 145 1, 145 3 (C-3′, 4′), 156.8, 157 8 (C-5, 7, 8a), 174.4 (COOH) (Found. C, 56 07, H, 5 29 $C_{17}H_{16}O_8 \cdot H_2O$ requires: C, 55 74, H, 4.95%)

Methylation of **1** A mixture of **1** (63 mg), Me_2SO_4 (0 5 ml) and anhyd. K_2CO_3 (600 mg) in dry Me_2CO (10 ml) was refluxed for 3 hr After removal of the inorganic salts by filtration, the reaction mixture was concd, and the residue was subjected to CC over silica gel Elution with C_6H_6–Me_2CO (17 1) yielded the pentamethylate (**1a**) (49 mg) as an amorphous powder, $[\alpha]_D^{20}$ −38 6° ($CHCl_3$, *c* 0 76). EIMS *m/z*. 418 (M$^+$), 400 [M−H_2O]$^+$, 327 [M−H_2O−CH_2COOMe]$^+$, 250, 239, 207, 180, 166, 151 ^{1}H NMR ($CDCl_3$) δ2 35 (1H, *dd*, *J* = 11, 16 Hz, H-1″), 3 06 (1H, *dd*, *J* = 4, 16 Hz, H-1″), 3 56 (1H, *dd*, *J* = 4, 11 Hz, H-4), 3 75, 3 78, 3 82, 3 90 3 93 (each 3H, *s*, OMe), 4 04 (1H, *s*, H-3), 4 99 (1H, *s*, H-2), 6 13, 6.21 (each 1H, *d*, *J* = 2 Hz, H-6, 8), 6 86–7 29 (3H in total, *m*, B-ring H) (Found C, 62 94, H, 6 48 $C_{22}H_{26}O_8$ requires C, 63 15, H, 6.26%)

Benzoylation of **1a** Compound **1a** (25 mg) was treated with benzoyl chloride (1 ml) and dry pyridine (1 ml) at room temp. overnight. The reaction mixture was worked-up as usual, and chromatographed over silica gel Elution with *n*-hexane–EtOAc (4:1) afforded the monobenzoate (**1b**) (21 mg) as an amorphous powder, $[\alpha]_D^{20}$ −144 1° ($CHCl_3$, *c* 0 85) EIMS *m/z*: 522 [M]$^+$, 400 [M−PhCOOMe], 327 CD (MeOH, *c* 1.38 × 10^{-5}) $[\theta]^{20}$ × 10^3 (nm) −12.2 (275) (trough), −5 7 (256) (peak), −8.5 (233) (trough), −3 0 (297) (peak), −18 2 (206) (trough). ^{1}H NMR ($CDCl_3$) δ2 48 (1H, *dd*, *J* = 11, 16 Hz, H-1″), 3 13 (1H, *dd*, *J* = 4, 16 Hz, H−1″), 3 59 (1H, *m*, H-4), 3.62, 3 80, 3 81, 3 83 (15 H in total, each *s*, OMe), 5 18 (1H, *s*, H-2), 5.51 (1H, *s*, H-3), 6 13, 6 26 (each 1H, *d*, *J* = 2 Hz, H-6, 8), 6 76–7 04 (3H in total, *m*, B-ring H), 7 24–7 94 (5H in total, aromatic H). (Found C, 66 33; H, 5 84 $C_{29}H_{30}O_9$ requires C, 66 65, H, 5 79%).

4β-Carboxymethyl-(−)-epicatechin potassium and sodium salts (**2**) An amorphous powder, $[\alpha]_D^{24}$ +22 9° (Me_2CO, *c* 0.56) Negative FABMS *m/z* 347 [M−K or M−Na]$^-$ Positive FABMS *m/z*· 387, 371 (each [M+H]$^+$), 349 IR ν_{max}^{KBr} cm^{-1}· 3400 (OH), 1600, 1510 (C=C), 1550, 1400 (COO$^-$) ^{1}H NMR (Me_2CO-d_6 + D_2O). δ2 38, 2 68 (each 1H, *dd*, *J* = 5, 16 Hz, H-1″), 3.34 (1H, *t*, *J* = 5 Hz, H-4), 3 90 (1H, *s*, H-3), 4 89 (1H, *s*, H-2), 5.92, 6.00 (each 1H, *d*, *J* = 2 Hz, H-6, 8), 6 81 (1H, *d*, *J* = 8 Hz, H-5′), 6.92 (1H, *dd*, *J* = 2, 8 Hz, H-6′), 7.08 (1H, *d*, *J* = 2 Hz, H-2′) ^{13}C NMR (Me_2CO-d_6 + D_2O) δ36 3 (C-4), 44 5 (C-1″), 71 1 (C-3), 75 3 (C-2), 96 7, 97 2 (C-6, 8), 104 2 (C-4a), 115.1 (C-2′), 115 8 (C-5′), 119.2 (C-6′), 131 9 (C-1′), 145 0, 145 2 (C-3′, 4′), 156 1, 157.3, 158 1 (C-5, 7, 8a) 174 1 (COO).

Methylation of **2** A mixture of **2** (40 mg), Me_2SO_4 (0 2 ml) and dry K_2CO_3 (400 mg) in dry Me_2CO (7 ml) was refluxed for 3 hr The reaction mixture was treated as for **1** to furnish the pentamethylate, which was shown to be identical with **1a**.

Acid treatment of **2** Compound **2** (20 mg) in 0 5 M HCl (5 ml) was heated in a water bath for 1 hr The reaction mixture was extracted with EtOAc, and the EtOAc layer, after removal of the solvent by evapn, was chromatographed over MCI-gel CHP 20P[H_2O–MeOH(1·0→1 1)] to yield **1**.

4β-Carboxymethyl-(−)-epicatechin methyl ester (**3**). An amorphous powder, $[\alpha]_D^{24}$ −58 3° (Me_2CO, *c* 0 7). Negative FABMS *m/z*· 361 [M−H]$^-$. ^{1}H NMR (Me_2CO-d_6) δ2 51 (1H, *dd*, *J* = 11, 16 Hz, H-1″), 3 05 (1H, *dd*, *J* = 4, 16 Hz, H-1″), 3.45 (1H, *dd*, *J* = 4, 11 Hz, H-4), 3 68 (3H, *s*, COOMe), 3 94 (1H, *s*, H-3), 4 91 (1H, *s*, H-2), 5 93, 6 05 (each 1H, *d*, *J* = 2 Hz, H-6, 8), 6 79 (1H, *d*, *J* = 8 Hz, H-5′), 6 88 (1H, *dd*, *J* = 2, 8 Hz, H-6′), 7 07 (1H, *d*, *J*

= 2 Hz, H-2') ^{13}C NMR (Me_2CO-d_6) δ 36.2 (C-4), 39.1 (C-1''), 51.7 (OMe), 70.1 (C-3), 75.3 (C-2), 95.8, 96.5 (C-6, 8), 102.5 (C-4a), 115.3 (C-2'), 115.5 (C-5'), 119.3 (C-6'), 132.0 (C-1'), 145.3, 145.4 (C-3', 4'), 157.0, 158.0 (C-5, 7, 8a), 173.2 (COO) (Found: C, 56.88; H, 5.23. $C_{18}H_{18}O_8 \cdot H_2O$ requires: C, 56.88; H, 5.30%)

Formation of **3**. A mixture of **1** (50 mg) and *p*-toluenesulphonic acid (5 mg) in MeOH (5 ml) was refluxed for 2 hr. The reaction mixture was concd. under red. pres. to dryness, and the residue was chromatographed over MCI-gel CHP 20P [H_2O–MeOH (1:0→1:1)] to afford **3** (32 mg).

Epicatechin-(4β→8)-4β-carboxymethylepicatechin (**4**). A tan amorphous powder, $[\alpha]_D^{24}$ +41.7° (Me_2CO, *c* 0.51). Negative FABMS *m/z*: 635 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1705 (C=O), 1600, 1510 (C=C). ^{1}H NMR (Me_2CO-d_6 + D_2O) δ: 2.43 (1H, *dd*, *J* = 10, 16 Hz, H-1''''), 3.06 (1H, *dd*, *J* = 4, 16 Hz, H-1''''), 4.01 (1H, *s*, H-3), 4.10 (1H, *s*, H-3''), 4.73 (1H, *s*, H-4), 4.99 (1H, *s*, H-2''), 5.08 (1H, *s*, H-2), 6.02 (3H in total, *m*, A-ring H), 6.65–7.18 (3H in total, *m*, B-ring H). ^{13}C NMR (Me_2CO-d_6 + D_2O) δ: 36.4 (C-4''), 36.9 (C-4), 40.0 (C-1''''), 69.6 (C-3''), 72.7 (C-3), 75.2 (C-2), 76.8 (C-2''), 95.9, 96.4, 97.5 (C-6, 8, 6''), 103.3 (C-4a, 4a''), 106.9 (C-8''), 115.0, 115.5, 115.7 (C-2', 5', 2'''', 5''''), 131.6, 132.0 (C-1', 1''''), 145.1, 145.2, 145.4 (C-3', 4', 3'''', 4''''), 154.0, 156.3, 157.5, 158.1 (C-5, 7, 8a, 5'', 7'', 8a''), 175.7 (COOH) (Found: C, 55.28; H, 4.96. $C_{32}H_{28}O_{14} \cdot 3H_2O$ requires: C, 55.65; H, 4.96%)

Thiolytic degradation of **4**. A mixture of **4** (20 mg), benzylmercaptan (1 ml) and HOAc (2 ml) in EtOH (5 ml) was refluxed for 3 hr with stirring. The reaction mixture was concd. under red. pres. to give an oily residue, which was chromatographed over Sephadex LH-20. Elution with Me_2CO afforded the thioether, which was subsequently purified by Sephadex LH-20 with H_2O–MeOH (1:4) to give (−)-epicatechin 4β-benzylthioether (**9**) (6 mg) as an amorphous powder, $[\alpha]_D^{24}$ −29.7° (Me_2CO, *c* 0.39). Further elution with Me_2CO yielded **1** (7 mg).

Epicatechin-(4β→8)-4β-carboxymethylepicatechin potassium and sodium salt (**5**). A tan amorphous powder, $[\alpha]_D^{24}$ +65.3° (Me_2CO, *c* 0.58). Negative FABMS *m/z*: 635 $[M-K$ or $M-Na]^-$. Positive FABMS *m/z*: 675, 659 (each $[M+H]^+$), 637. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1600, 1520 (C=C). ^{1}H NMR (Me_2CO-d_6 + D_2O) δ: 2.42, 2.86 (1H, *dd*, *J* = 5, 16 Hz, H-1''''), 3.43 (1H, *t*, *J* = 5 Hz, H-4''), 4.00 (1H, *s*, H-4), 5.01 (1H, *br s*, H-2''), 5.11 (1H, *s*, H-2), 5.91–6.04 (3H in total, *m*, A-ring H), 6.70–7.16 (6H in total, *m*, B-ring H). ^{13}C NMR (Me_2CO-d_6 + D_2O) δ: 36.8 (C-4, 4''), 44.1 (C-1''''), 70.3 (C-3''), 72.7 (C-3), 75.3 (C-2), 76.6 (C-2''), 95.8, 96.3, 97.9 (C-6, 8, 6''), 104.3 (C-4a, 4a''), 106.9 (C-8''), 114.9, 115.2, 115.7, 115.8 (C-2', 5', 2'''', 5''''), 119.2 (C-6', 6''''), 131.7, 132.0 (C-1', 1''''), 144.9, 145.1, 145.3 (C-3', 4', 3'''', 4''''), 153.6, 156.1, 156.3, 157.3, 157.7 (C-5, 7, 8a, 5'', 7'', 8a''), 176.1 (COO).

Thiolytic degradation of **5**. A mixture of **5** (30 mg), benzylmercaptan (1 ml) and HOAc (1 ml) in EtOH (5 ml) was refluxed for 3 hr with stirring. The reaction mixture was worked up as for **4** to furnish **9** (8 mg) and **1** (12 mg).

Epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin (**6**). A tan amorphous powder, $[\alpha]_D^{24}$ +179.2° (Me_2CO; *c* 0.73). Negative FABMS *m/z*: 1153 $[M-H]^-$. The ^{1}H NMR spectrum was complicated by rotational isomerism. ^{13}C NMR (Me_2CO-d_6 + D_2O) δ: 37.2, 37.6 (C-4, 4', 4''), 66.8, 71.1, 72.4, 73.1 (C-3, 3', 3'', 3'''), 76.7 (C-2, 2', 2''), 79.2 (C-2''') (Found: C, 59.83; H, 4.68. $C_{60}H_{50}O_{24} \cdot 3H_2O$ requires: C, 59.60; H, 4.67%).

Partial thiolytic degradation of **6**. A mixture of **6** (148 mg), benzylmercaptan (3 ml) and HOAc (2 ml) in EtOH (15 ml) was refluxed for 3 hr with stirring. The reaction mixture was worked-up as for **4**, and chromatographed over Sephadex LH-20 [EtOH and H_2O–MeOH (2:3→1:4)] to afford (−)-epicatechin 4β-benzylthioether (**9**) (12 mg), procyanidin B-5 4''β-benzylthioether (**10**) (7 mg), procyanidin B-2 4''β-benzylthioether (**11**) (3 mg), (−)-epicatechin (**12**) (4 mg), procyanidin B-5 (**13**) (10 mg), and epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin (**14**) (19 mg).

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