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# FLAVAN-3-OL AND PROANTHOCYANIDIN ALLOSIDES FROM *DAVALLIA DIVARICATA**

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**Key Word Index**—*Davallia divaricata*; Davalliaceae; fern; flavan-3-ol; proanthocyanidins; allosides; procyanidins; condensed tannin.

**Abstract**—Four flavan-3-ol glycosides and three proanthocyanidin glycosides have been isolated from the fern, *Davallia divaricata*, together with procyanidins B-1 and B-2 and a trimeric procyanidin. All of these glycosides have been shown to possess an allopyranose moiety. The structures were elucidated on the basis of chemical and spectroscopic evidence.

## INTRODUCTION

Although tannins have been found in many species of higher plants, little is known of their occurrence in ferns, horsetails, lycopods, algae, liverworts or mosses. However, gallotannins have been reported from the freshwater green alga *Spirogyra* sp. [2] and some proanthocyanidins from the ferns *Arachniodes pseudo-arista* and *A. aristata* [3]. In the course of a recent survey of various plant sources for tannins, encountered a large accumulation of condensed tannins in the rhizomes of the subtropical fern, *Davallia divaricata* Blume (Davalliaceae), which is used as a crude drug in China and Taiwan for the relief of joint pain. Subsequent large-scale extraction has now led to the isolation of seven new flavan-3-ol and proanthocyanidin allosides, together with dimeric and trimeric proanthocyanidins. This paper reports on the isolation and characterization of these compounds.

## RESULTS AND DISCUSSION

Repeated chromatography of the aqueous acetone extract of the rhizome on Sephadex LH-20 and various reverse-phase gels afforded four flavan-3-ol glycosides (**1**–**4**) and three proanthocyanidin glycosides (**5**–**7**), together with procyanidins B-2 (**8**) and B-1 (**9**) [4–6] and a trimeric procyanidin (**10**). Among them, compounds **1** and **10** were found to be identical with (−)-epicatechin 3-*O*-β-D-allopyranoside [7] and epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin [8], respectively, by comparisons of their physical and spectral data with literature values.

Compound **2** gave an orange colour with anisaldehyde–sulphuric acid, which is characteristic of flavan-3-ol derivatives. The $^1$H and $^{13}$C NMR spectra of **2**, being similar to those of catechin, revealed the presence of a flavan-3-ol skeleton with a 2,3-*trans* configuration. The existence of a sugar moiety was indicated by an anomeric proton resonance (δ4.89) and by six aliphatic carbon resonances (Table 1). Ordinary phenol methylation with diazomethane yielded the tetramethyl ether (**2a**), which, on further acid hydrolysis with 1 M HCl, afforded 5,7,3′,4′-tetra-*O*-methyl (+)-catechin and D-allose, indicating that the D-allose moiety is attached to the catechin C-3 hydroxyl group. The mode of the sugar linkage was concluded to be β from the anomeric coupling constant value (*d*, $J = 8$ Hz). On the basis of these observations, **2** was established as (+)-catechin 3-*O*-β-D-allopyranoside.

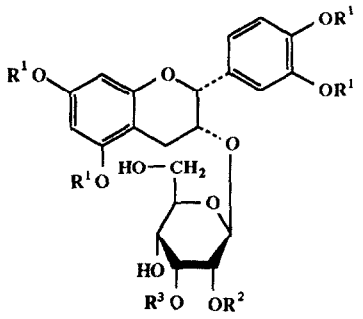
The $^1$H and $^{13}$C NMR spectra of **3** and **4**, being similar to each other, were closely correlated with those of **1**, showing the presence of epicatechin and sugar moieties. Additional signals of aromatic ABX [δ6.87 (1H, *d*, $J = 8$ Hz), 7.41 (1H, *dd*, $J = 2$, 8 Hz) and 7.47 (1H, *d*, $J = 2$ Hz) in **3**; δ6.92 (1H, *d*, $J = 8$ Hz), 7.58 (1H, *d*, $J = 2$ Hz) and 7.62 (1H, *dd*, $J = 2$, 8 Hz) in **4**] and of a methoxyl (δ3.68 in **3**; δ3.90 in **4**), as well as an ester carboxyl resonance (Table 1), suggested that either a vanillyl or an isovanillyl moiety is present in each molecule. On acid hydrolysis, both **3** and **4** yielded vanillic acid, while methylation of **3** and **4** followed by alkaline methanolysis gave in each case 5,7,3′,4′-tetra-*O*-methyl (−)-epicatechin 3-*O*-β-D-allopyranoside.

In the $^1$H NMR spectrum of **3**, one methine signal appeared downfield at δ4.64 as a double doublet ($J = 3$, 8 Hz), while **4** exhibited the lowfield signal at δ5.75 as a triplet ($J = 3$ Hz). These signals could be assigned to H-2 and H-3 of the allose moiety, respectively, on the basis of $^1$H–$^1$H COSY spectral examinations. From these chemical and spectral evidence, **3** and **4** were characterized as (−)-epicatechin 3-*O*-β-D-(2″-*O*-vanillyl)- and 3-*O*-β-D-(3″-*O*-vanillyl)allopyranosides, respectively.

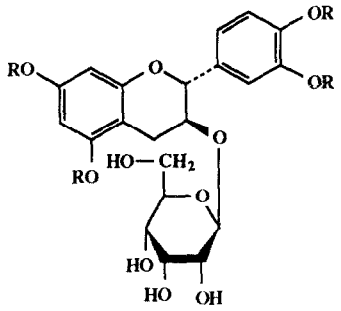
The $^1$H NMR spectrum of **5** resembled those of **1** and procyanidin B-2 (**8**). The negative FABMS showed the $[M-H]^-$ peak at *m/z* 739, corresponding to the monohexoside of a dimeric procyanidin. The presence of the allose moiety was deduced from $^{13}$C NMR analysis,

*Part 74 in the series 'Tannins and Related Compounds'. For Part 73 see ref. [1].

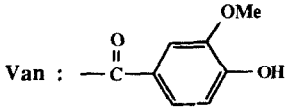
‡Author to whom correspondence should be addressed.

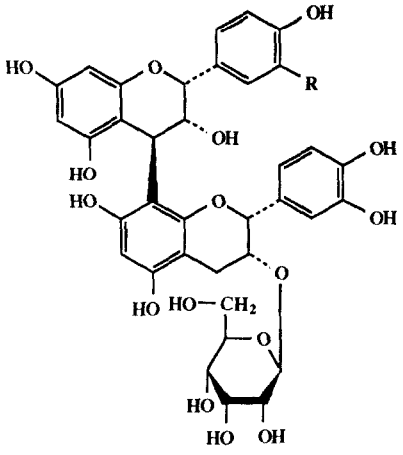


| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| 1 | H | H | H |
| 1a | Me | H | H |
| 3 | H | Van | H |
| 4 | H | H | Van |

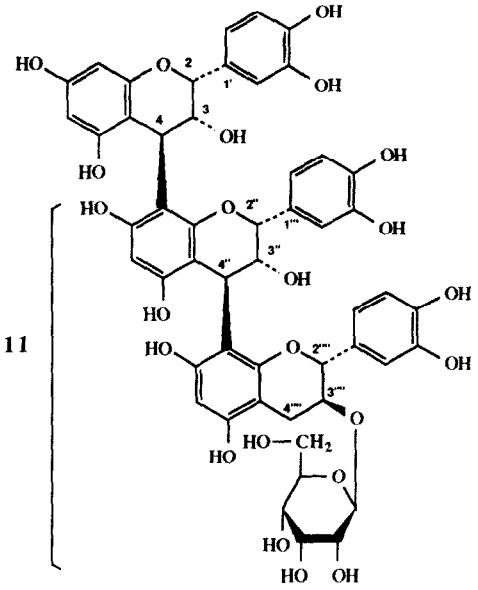


| | |
|---|---|
| 2 | R = H |
| 2a | R = Me |





| | |
|---|---|
| 5 | R = OH |
| 6 | R = H |



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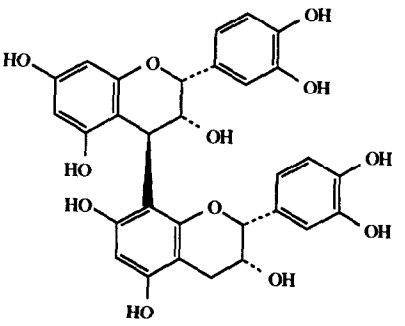
which showed an aliphatic signal pattern similar to those found in **1** and **2**. Methylation followed by acid hydrolysis yielded octa-*O*-methyl procyanidin B-2 and allose. Furthermore, acid catalysed degradation in the presence of benzylmercaptan [5] gave (−)-epicatechin 4$\beta$-benzylthioether (formed from the upper half) and **1** (from the lower half), indicating that the allose moiety is bound to the C-3 hydroxyl group in the lower flavan unit. From these findings, **5** was determined as procyanidin B-2 3″-*O*-$\beta$-D-allopyranoside.

The negative FABMS of **6** showed the $[M-H]^-$ peak at *m/z* 723, which is 16 mass units less than that of **5**. The aliphatic region in the $^{13}C$ NMR spectrum was closely correlated with that of **5**, showing the presence of the allose moiety. The $^1H$ NMR spectrum was also similar to that of **5**, but differed only in the observation of $A_2B_2$-type aromatic signals instead of ABX-type signals, suggesting the presence of an epiafzelechin unit. Acid catalysed degradation in the presence of benzylmercaptan furnished (−)-epiafzelechin 4$\beta$-benzylthioether [9] and **1**. The location and the configuration of the interflavanoid linkage were concluded to be C(4$\beta$)–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 is almost identical with those observed in **5**. On the basis of these chemical and spectral findings, **6** was characterized as epiafzelechin-(4$\beta$→8)-epicatechin 3-*O*-$\beta$-D-allopyranoside.



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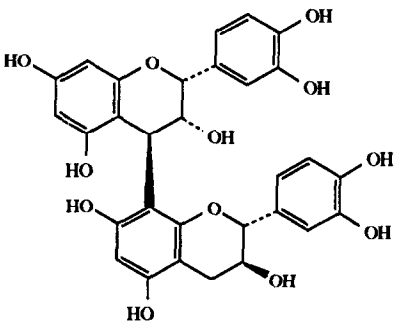

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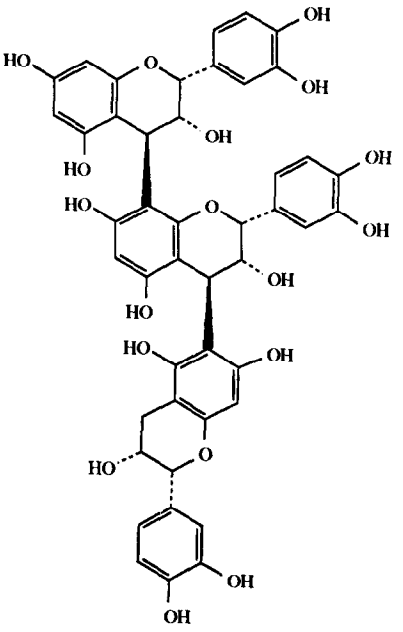

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The monohexosyl trimeric constitution of **7** was confirmed by negative FABMS ($[M-H]^-$: *m/z* 1027). The $^1H$ and $^{13}C$ NMR spectra, measured at room temperature, were complicated by conformational isomerism. However, $^{13}C$ resonances arising from the major conformer confirmed the presence of the allose moiety in **7**. Partial thiolytic degradation of **7** furnished procyanidin B-2 4″-benzylthioether and a dimeric procyanidin glycoside (**11**), together with **2** and (−)-epicatechin 4β-benzylthioether. The structure of compound **11** followed from a $^1H$ NMR examination; the spectrum was similar to that of **5** except for the coupling patterns of lower flavan H-2, H-3 and H-4. Accordingly, **7** was characterized as epicatechin-(4β→8)-epicatechin-(4β→8)-catechin 3-*O*-β-D-allopyranoside. Recently, Murakami *et al.* isolated (−)-epicatechin 3-*O*-β-D-allopyranoside (**1**) from the fronds of the same plant, but this is the first isolation of proanthocyanidin glycosides from ferns. The presence of a sugar moiety only in the lower terminal unit in all the proanthocyanidins isolated here suggests that biosynthetic elongation of the proanthocyanidin chains occurs in the upper units.

## EXPERIMENTAL

Details of the instruments and chromatographic conditions used in this work are similar to those described in [1].

*Plant material.* The rhizomes of *Davallia divaricata* were collected at San-Chi-Mon near Ping-tung City, Taiwan. Voucher specimens are deposited in 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 CC over Sephadex LH-20 using $H_2O$ with increasing amounts of MeOH (1:0 → 0:1) to give 4 fractions; frs 1 (34.5 g), 2 (6.2 g), 3 (15.8 g) and 4 (13.0 g). Fraction 1 consisted mainly of **1**, which was cryst. from $H_2O$ to yield 22.9 g of pure sample. The mother liquor was chromatographed over MCI-gel CHP 20P [$H_2O$–MeOH (1:0 → 4:1)] and monitored by HPLC to furnish **2** (1.6 g). Fraction 2 was chromatographed over MCI-gel CHP 20P [$H_2O$–MeOH (1:0 → 1:4)] to afford a further three fractions (2-1–2-3). Repeated CC of fr. 2-1 over Sephadex LH-20 (EtOH) and Bondapak $C_{18}$ Porasil B [$H_2O$–MeOH (1:0 → 4:1)] furnished **7** (148 mg). CC of fr. 2–2 over Sephadex LH-20 with EtOH gave two fractions, which were each purified by CC on Bondapak $C_{18}$ Porasil B [$H_2O$–MeOH (1:0 → 4:1)] to yield **5** (433 mg) and **6** (53 mg). Fr. 2–3 was chromatographed over Sephadex LH-20 (EtOH) and Prep Pak-500/$C_{18}$ [$H_2O$–MeOH (1:4 → 4:1)] to furnish **3** (822 mg). Fraction 3 was fractionated by CC on Sephadex LH-20 eluted with EtOH into further five fractions (3-1–3-5). Repeated CC of fr. 3-4 over Sephadex LH-20 ($Me_2CO$) and MCI-gel CHP 20P [$H_2O$–MeOH (1:0 → 1:4)] afforded **4** (247 mg).

*(−)-Epicatechin 3-O-β-D-allopyranoside* (**1**) Colourless needles ($H_2O$), mp 165–168°, $[\alpha]_D^{20}$ −32.5° (MeOH; *c* 0.67). Negative FABMS *m/z*: 451 $[M-H]^-$. $^1H$ NMR ($Me_2CO$-$d_6$ + $D_2O$): δ2.61 (1H, *dd*, *J* = 16, 6 Hz, H-4), 2.62 (1H, *dd*, *J* = 5, 16 Hz, H-4), 3.3–3.9 (5H, *m*, sugar -H), 4.11 (1H, *t*, *J* = 2 Hz, allosyl H-3), 4.42 (1H, *m*, H-3), 4.84 (1H, *d*, *J* = 8 Hz, anomeric H), 5.17 (1H, *d*, *J* = 2 Hz, H-2), 5.95, 6.05 (each 1H, *d*, *J* = 2 Hz, H-6 and 8), 6.73 (1H, *d*, *J* = 8 Hz, H-5′), 6.78 (1H, *dd*, *J* = 2, 8 Hz, H-6′), 7.16 (1H, *d*, *J* = 2 Hz, H-2′). Methylation of **1** gave 5,7,3′,4′-tetra-*O*-methyl (−)-epicatechin 3-alloside, which was identical with the methanolysis product of **3a** (see below).

*(+)-Catechin 3-O-β-D-allopyranoside* (**2**). A white amorphous powder, $[\alpha]_D^{24}$ −169.0° ($Me_2CO$; *c* 0.72). Negative FABMS (*m/z*): 451 $[M-H]^-$, 289 $[M-\text{allosyl}]^-$. $^1H$ NMR ($Me_2CO$-$d_6$ + $D_2O$): δ2.68 (2H, *d*, *J* = 5 Hz, H-4), 3.2–3.9 (5H, *m*, sugar-H), 4.07 (1H, *t*, *J* = 3 Hz, allosyl H-3), 4.36 (1H, *q*, *J* = 5 Hz, H-3), 4.89

Table 1. $^{13}C$ NMR data for flavan-3-ol allosides (25.05 MHz, TMS)

| | C | 1* | 2† | 3† | 4† |
|---|---|---|---|---|---|
| | 2 | 76.6 | 79.9 | 78.0 | 78.3 |
| | 3 | 72.2 | 73.9 | 74.1 | 73.9 |
| | 4 | 22.9 | 23.4 | 24.4 | 24.6 |
| | 5 | 156.1 | 157.1 | 157.1 | 157.1 |
| | 6 | 93.9 | 96.2 | 96.0 | 96.1 |
| | 7 | 156.4 | 157.5 | 157.4 | 157.7 |
| | 8 | 95.0 | 95.2 | 95.4 | 95.5 |
| | 4a | 99.4 | 99.5 | 99.3 | 99.9 |
| | 8a | 155.0 | 56.1 | 156.5 | 156.7 |
| | 1′ | 129.5 | 132.0 | 131.0 | 131.4 |
| | 2′ | 114.5 | 114.7 | 115.1 | 115.1 |
| | 3′ | 144.1‡ | 145.3‡ | 144.8‡ | 144.9‡ |
| | 4′ | 144.2‡ | 145.4‡ | 145.0‡ | 145.2‡ |
| | 5′ | 115.1 | 115.7 | 115.9 | 116.0 |
| | 6′ | 118.4 | 119.1 | 119.9 | 120.0 |
| | 1 | 98.3 | 101.1 | 97.3 | 101.2 |
| | 2 | 70.5 | 71.6 | 73.2 | 70.9 |
| Allose | 3 | 71.4 | 72.1 | 70.3 | 74.8 |
| moiety | 4 | 67.5 | 68.6 | 68.1 | 68.0 |
| | 5 | 74.2 | 75.0 | 75.0 | 75.9 |
| | 6 | 61.5 | 62.9 | 62.8 | 63.0 |
| | 1 | | | 122.3 | 123.1 |
| | 2 | | | 113.3 | 113.6 |
| | 3 | | | 152.0 | 151.9 |
| Vanillyl | 4 | | | 147.9 | 147.9 |
| moiety | 5 | | | 115.5 | 115.5 |
| | 6 | | | 125.0 | 124.9 |
| | OMe | | | 56.3 | 56.3 |
| | –COO– | | | 166.3 | 166.4 |

*Measured in DMSO-$d_6$.
†Measured in $Me_2CO$-$d_6$.
‡Assignment may be interchanged in each column.

(1H, *d*, $J = 8$ Hz, anomeric H), 5.07 (1H, *d*, $J = 5$ Hz, H-2), 5.92, 6.02 (each 1H, *d*, $J = 2$ Hz, H-6 and H-8), 6.69 (1H, *dd*, $J = 2$, 8 Hz, H-6′), 6.79 (1H, *d*, $J = 8$ Hz, H-5′), 6.93 (1H, *d*, $J = 2$ Hz, H-2′). (Found: C, 53.09; H, 5.78. $C_{21}H_{24}O_{11} \cdot 3/2H_2O$ requires: C, 52.61; H, 5.68%).

*Methylation of* **2**. A soln of **2** (160 mg) in MeOH (5 ml) was treated overnight with ethereal $CH_2N_2$ at 0°. The concd mixture was subjected to CC over silica gel [$CHCl_3$–MeOH (30:1–15:1)] to yield the tetramethyl ether (**2a**) (85 mg) as a white amorphous powder, $[\alpha]_D^{24} -146.6°$ ($CHCl_3$; *c* 0.64). FDMS (*m/z*): 509 (M + H)$^+$, 328 [M − allosyl]$^+$. $^1H$ NMR ($CDCl_3$): δ2.6–2.9 (2H, *m*, H-4), 3.2–3.9 (5H, *m*, sugar-H), 3.76, 3.77, 3.84, 3.86 (each 3H, *s*, OMe), 4.19 (1H, *br s*, allosyl H-3), 4.30 (1H, *m*, H-3), 4.80 (1H, *d*, $J = 8$ Hz, anomeric H), 5.05 (1H, *d*, $J = 7$ Hz, H-2), 6.08, 6.16 (each 1H, *d*, $J = 2$ Hz, H-6 and H-8), 6.72–6.96 (3H, *m*, B-ring H). (Found: C, 57.19; H, 6.70. $C_{25}H_{32}O_{11} \cdot H_2O$ requires: C, 57.03; H, 6.51%).

*Acid hydrolysis of* **2a**. A soln of **2a** (48 mg) in 1 M HCl [$H_2O$–$Me_2CO$ (1:1)] (10 ml) was refluxed for 4 hr. The reaction mixture was concd to give a white ppt, which was treated with $CHCl_3$. The $CHCl_3$-soluble portion cryst. from MeOH to give an aglycone (14 mg), which was identical with 5,7,3′,4′-tetra-*O*-methyl (+)-catechin. The filtrate was neutralized with Amberlite IRA-400 ($OH^-$ form) resin, concd and chromatographed over silica gel with $CHCl_3$–MeOH–$H_2O$ (14:6:1) to yield D-allose as a white amorphous powder, $[\alpha]_D^{26} + 13.6°$ ($H_2O$; *c* 1.0), $R_f$ 0.46 [cellulose, EtOAc–pyridine–$H_2O$–HOAc (5:5:3:1)].

*(−)-Epicatechin 3-O-β-D-(2″-O-vanillyl)allopyranoside* (**3**). A white amorphous powder, $[\alpha]_D^{24} -88.1°$ ($Me_2CO$; *c* 1.0). Negative FABMS *m/z*: 601 [M − H]$^-$. $^1H$ NMR ($Me_2CO$-$d_6 + D_2O$): δ2.64 (1H, *dd*, $J = 6$, 16 Hz, H-4), 2.88 (1H, *dd*, $J = 5$, 16 Hz, H-4), 3.5–3.9 (4H, *m*, sugar-H), 4.39 (1H, *br s*, allosyl H-3), 4.49 (1H, *m*, H-3), 4.64 (1H, *dd*, $J = 3$, 8 Hz, allosyl H-2), 5.12 (1H, *d*, $J = 2$ Hz, H-2), 5.20 (1H, *d*, $J = 8$ Hz, anomeric H), 5.87, 6.00 (each 1H, *d*, $J = 2$ Hz, H-6 and H-8), 6.66 (1H, *d*, $J = 8$ Hz, H-5′), 6.83 (1H, *dd*, $J = 2$, 8 Hz, H-6′), 6.87 (1H, *d*, $J = 8$ Hz, vanillyl H-5), 7.14 (1H, *d*, $J = 2$ Hz, H-2′), 7.41 (1H, *dd*, $J = 2$, 8 Hz, vanillyl H-6), 7.47 (1H, *d*, $J = 2$ Hz, vanillyl H-2). (Found: C, 56.07; H, 5.29. $C_{29}H_{30}O_{14} \cdot H_2O$ requires: C, 56.13; H, 5.20%).

*Acid hydrolysis of* **3**. A soln of **3** (20 mg) in 5% $H_2SO_4$ (2 ml) was heated at 100° for 4 hr. After cooling, the reaction mixture was extracted × 3 with EtOAc and the concd extract chromatographed on MCI-gel CHP 20P [$H_2O$–MeOH (1:0–1:1)] to afford vanillic acid (3.0 mg) as colourless needles, mp 210–212°. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3500 (OH), 3000 (COOH), 1680 (CO), 1600, 1520 (aromatic ring).

*Methylation of* **3**. A mixture of **3** (60 mg), $Me_2SO_4$ (0.4 ml) and dry $K_2CO_3$ (600 mg) in dry $Me_2CO$ (20 ml) was refluxed for 2 hr. The concd mixture was subjected to CC over silica gel. Elution with $CHCl_3$–MeOH (50:1–40:1) yielded the hexamethyl ether (**3a**) (38 mg) as a white amorphous powder. $[\alpha]_D^{20} -86.0°$ ($CHCl_3$; *c* 0.66). FDMS *m/z*: 672 (M)$^+$. $^1H$ NMR ($CDCl_3$): δ2.60–2.96 (2H, *m*, H-4), 3.6–3.9 (4H, *m*, sugar-H), 3.66, 3.68, 3.84, 3.88, 3.94 (18H in total, each *s*, 6 × OMe), 4.38 (2H, *br s*, H-3 and allosyl H-3), 4.82 (1H, *dd*, $J = 3$, 8 Hz, allosyl H-2), 5.00 (1H, *br s*, H-2), 5.10 (1H, *d*, $J = 8$ Hz, anomeric H), 5.93, 6.01 (each 1H, *d*, $J = 2$ Hz, H-6 and H-8), 6.73 (1H, *d*, $J = 8$ Hz, H-5′), 6.75 (1H, *d*, $J = 8$ Hz, vanillyl H-5), 6.92 (1H, *dd*, $J = 2$, 8 Hz, vanillyl H-5), 7.12 (1H, *d*, $J = 2$ Hz, H-2′), 7.31 (1H, *dd*, $J = 2$, 8 Hz, vanillyl H-6), 7.34 (1H, *d*, $J = 2$ Hz, vanillyl H-2). (Found: C, 59.21; H, 6.00. $C_{35}H_{42}O_{14} \cdot H_2O$ requires: C, 59.65; H, 6.29%).

*Methanolysis of* **3a**. A soln of **3a** (15 mg) in 0.2% NaOMe–MeOH (5 ml) was left at room temp. for 3 hr, the soln neutralized with Amberlite IR-120B ($H^+$ form) resin, and the products separated by CC over silica gel [$CHCl_3$–MeOH (40:1–20:1)] to give 5,7,3′,4′-tetra-*O*-methyl (−)-epicatechin 3-*O*-β-D-allopyranoside (7.8 mg) as a white amorphous powder, $[\alpha]_D^{23} -25.6°$ ($CHCl_3$; *c* 1.1). $^1H$ NMR ($CDCl_3$): δ2.86 (2H, *d*, $J = 5$ Hz, H-4), 3.2–3.9 (5H, *m*, sugar-H), 3.77, 3.80, 3.89 (12 H in total, each *s*, OMe), 4.17 (1H, *br s*, allosyl H-3), 4.39 (1H, *m*, H-3), 4.72 (1H, *d*, $J = 8$ Hz, anomeric H), 5.05 (1H, *s*, H-2), 6.11, 6.19 (each 1H, *d*, $J = 2$ Hz, H-6 and H-8), 6.84 (1H, *d*, $J = 8$ Hz, H-5′), 7.00 (1H, *dd*, $J = 2$, 8 Hz, H-6′), 7.15 (1H, *d*, $J = 2$ Hz, H-2′).

*(−)-Epicatechin 3-O-β-D-(3″-O-vanillyl)allopyranoside* (**4**). A white amorphous powder, $[\alpha]_D^{23} -66.9°$ ($Me_2CO$; *c* 0.77). Negative FABMS *m/z*: 601 [M − H]$^-$. $^1H$ NMR ($Me_2CO$-$d_6$): δ2.60 (1H, *dd*, $J = 6$, 16 Hz, H-4), 2.87 (1H, *dd*, $J = 5$, 16 Hz, H-4), 3.5–4.2 (5H, *m*, sugar-H), 3.90 (3H, *s*, OMe), 4.45 (1H, *m*, H-3), 4.98 (1H, *d*, $J = 8$ Hz, anomeric H), 5.21 (1H, *d*, $J = 2$ Hz, H-2), 5.75 (1H, *t*, $J = 3$ Hz, allosyl H-3), 5.94, 6.01 (each 1H, *d*, $J = 2$ Hz, H-6 and H-8), 6.74 (1H, *d*, $J = 8$ Hz, H-5′), 6.86 (1H, *dd*, $J = 2$, 8 Hz, H-6′), 6.92 (1H, *d*, $J = 8$ Hz, vanilloyl H-5), 7.17 (1H, *d*, $J = 2$ Hz, H-2′), 7.58 (1H, *d*, $J = 2$ Hz, vanilloyl H-2), 7.62 (1H, *dd*, $J = 2$, 8 Hz, vanilloyl H-6). (Found: C, 53.37; H, 5.35. $C_{29}H_{30}O_{14} \cdot 3H_2O$ requires: C, 53.05; H, 5.53%).

*Methylation of* **4**. Methylation of **4** gave the hexamethyl ether (**4a**) (28.3 mg) as a white amorphous powder. $[\alpha]_D^{20} -80.6°$ ($CHCl_3$; *c* 0.66). FDMS *m/z*: 672 (M)$^+$, 508 [M − dimethoxybenzoyl]$^+$. $^1H$ NMR ($CDCl_3$): δ2.6–3.0 (2H, *m*, H-4), 3.6–3.9 (5H, *m*, sugar-H), 3.76, 3.89, 3.93, 3.96 (18H in total, each *s*, OMe), 4.45 (1H, *m*, H-3), 4.85 (1H, *d*, $J = 8$ Hz, anomeric H), 5.08 (1H, *s*, H-2), 5.69 (1H, *t*, $J = 3$ Hz, allosyl H-3), 5.90, 6.11 (each 1H, *d*, $J = 2$ Hz, H-6 and H-8), 6.85 (1H, *d*, $J = 8$ Hz, H-5′), 6.90 (1H, *d*, $J = 8$ Hz,

vanillyl H-5), 7.00 (1H, *dd*, $J = 2, 8$ Hz, H-6'), 7.16 (1H, *d*, $J = 2$ Hz, H-2'), 7.54 (1H, *d*, $J = 2$ Hz, vanillyl H-2), 7.65 (1H, *dd*, $J = 2, 8$ Hz, vanillyl H-6). (Found: C, 58.11; H, 6.07. $C_{35}H_{42}O_{14} \cdot 2H_2O$ requires: C, 58.16; H, 6.42%).

*Methanolysis of* **4a**. Methanolysis of **4a** furnished 5,7,3',4'-tetra-*O*-methyl (−)-epicatechin 3-*O*-β-D-allopyranoside (7.5 mg).

*Procyanidin B-2 3''-O-β-D-allopyranoside* (**5**). A brown amorphous powder, $[\alpha]_D^{24} -8.7°$ ($Me_2CO$; *c* 0.98). Negative FABMS *m/z*: 739 $[M-H]^-$. $^1H$ NMR ($Me_2CO$-$d_6 + D_2O$): δ2.64 (1H, *dd*, $J = 6$, 16 Hz, H-4''), 2.91 (1H, *dd*, $J = 4$, 16 Hz, H-4''), 3.3–3.9 (5H, *m*, sugar-H), 4.00 (1H, *s*, H-3), 4.11 (1H, *t*, $J = 3$ Hz, allosyl H-3), 4.61 (1H, *m*, H-3''), 4.67 (1H, *s*, H-4), 4.90 (1H, *d*, $J = 8$ Hz, anomeric H), 5.10 (1H, *s*, H-2), 5.28 (1H, *br s*, H-2''), 6.00–6.06 (3H, *m*, A-ring H), 6.67–7.30 (6H, *m*, B-ring H). $^{13}C$ NMR ($Me_2CO$-$d_6 + D_2O$): δ24.5 (C-4''), 36.9 (C-4), 95.7, 96.3, 97.2 (C-6, C-8 and C-6''), 99.8 (C-4a and C-4a''), 107.2 (C-8''), 115.2, 115.5 (C-2', C-5', C-2''' and C-5'''), 119.2, 119.8 (C-6' and C-6'''), 130.6, 132.1 (C-1' and C-1'''), 144.7, 144.9, 145.1, 145.3 (C-3', C-4', C-3''' and C-4'''), 153.8, 155.4, 155.9, 157.4, 158.2 (C-5, C-7, C-8a, C-5'', C-7'' and C-8a''). The chemical shifts for the flavan C-ring and the sugar moiety are listed in Table 2. (Found: C, 52.65; H, 5.44. $C_{36}H_{36}O_{17} \cdot 9/2\ H_2O$ requires: C, 52.62; H, 5.52%).

*Methylation of* **5**. Methylation of **5** gave the octamethyl ether (**5a**) which was purified on silica gel [$CHCl_3$–MeOH (45:1–20:1)] as a white amorphous powder (32 mg) $[\alpha]_D^{23}$ −44.6° ($CHCl_3$; *c* 0.57). FDMS *m/z*: 853 $[M+H]^+$, 691 $[M+2H-allosyl]^+$. The $^1H$ NMR spectrum was complicated by conformational isomerism. (Found: C, 58.56; H, 6.19. $C_{44}H_{54}O_{17} \cdot 5/2H_2O$ requires: C, 58.72; H, 6.61%).

*Acid hydrolysis of* **5a**. A soln of **5a** (25 mg) in 1M HCl [$H_2O$–$Me_2CO$ (1:1)] (5 ml) was refluxed for 3 hr. The concd mixture was extracted with $CHCl_3$, and the concd $CHCl_3$ extract chromatographed on silica gel [$C_6H_6$–$Me_2CO$ (9:1)] to furnish procyanidin B-2 octamethyl ether (4.2 mg). The aq. layer, neutralized with 1 M KOH–MeOH, and concd to dryness gave allose [Rf. 0.46 on TLC cellulose, EtOAc–pyridine–$H_2O$–HOAc (5:5:3:1)].

*Thiolytic degradation of* **5**. A mixture of **5** (98 mg), benzylmercaptan (2 ml) and HOAc (3 ml) in EtOH (7 ml) was refluxed for 3 hr with stirring. The concd reaction mixture was chromatographed on 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 (44 mg). Further elution with $Me_2CO$ yielded (−)-epicatechin 3-*O*-β-D-allopyranoside (40 mg) (**1**).

*Epiafzelechin-(4β→8)-epicatechin 3-O-β-D-allopyranoside* (**6**). A brown amorphous powder, $[\alpha]_D^{24} + 2.6°$ ($Me_2CO$; *c* 0.77). Negative FABMS *m/z* 723 $[M-H]^-$. $^1H$ NMR [$Me_2CO$-$d_6 + D_2O$]: δ2.62 (1H, *dd*, $J = 6$, 16 Hz, H-4''), 2.88 (1H, *dd*, $J = 4$, 16 Hz, H-4''), 3.3–3.9 (5H, *m*, sugar-H), 4.00 (1H, *s*, H-3), 4.12 (1H, *t*, $J = 3$ Hz, allosyl H-3), 4.62 (1H, *m*, H-3''), 4.68 (1H, *s*, H-4), 4.90 (1H, *d*, $J = 8$ Hz, anomeric H), 5.17 (1H, *s*, H-2), 5.27 (1H, *br s*, H-2''), 6.00–6.04 (3H, *m*, A-ring H), 6.66 (1H, *d*, $J = 8$ Hz, H-5''''), 6.80 (2H, *d*, $J = 8$ Hz, H-3' and H-5'), 6.88 (1H, *dd*, $J = 2, 8$ Hz, H-6''''), 7.27 (2H, *d*, $J = 8$ Hz, H-2' and H-6''), 7.31 (1H, *d*, $J = 2$ Hz, H-2''''). $^{13}C$ NMR ($Me_2CO$-$d_6 + D_2O$): δ24.5 (C-4''), 36.9 (C-4), 95.7, 96.2, 97.2 (C-6, C-8 and C-6''), 99.8 (C-4a and C-4a''), 107.3 (C-8''), 115.5 (C-3', C-5', C-2''' and C-5'''), 119.8 (C-6'''), 129.1 (C-2''' and C-6'''), 130.6, 131.3 (C-1' and C-1'''), 144.9 (C-3''' and C-4'''), 153.8, 155.3, 155.9, 157.4, 158.1 (C-5, C-7, C-8a, C-5'', C-7'' and C-8a''). The chemical shifts for the flavan C-ring and the sugar moiety are listed in Table 2. (Found: C, 55.69; H, 5.47. $C_{36}H_{36}O_{16} \cdot 3H_2O$ requires: C, 55.52; H, 5.44%).

*Thiolytic degradation of* **6**. A mixture of **6** (25 mg), benzylmercaptan (1 ml) and HOAc (1 ml) in EtOH (4 ml) was refluxed for 4 hr with stirring. The reaction mixture was worked-up as for **5** to afford (−)-epiafzelechin 4β-benzylthioether (11 mg) and (−)-epicatechin 3-*O*-β-D-allopyranoside (10 mg).

Table 2. $^{13}C$ NMR data for procyanidin allosides (25.05 MHz, $Me_2CO$-$d_6 + D_2O$, TMS)

| | C | 5 | 6 | 7 |
|---|---|---|---|---|
| | 2 | 76.8 | 76.8 | 76.8 |
| | 3 | 72.5 | 72.5 | 73.1 |
| | 4 | 36.9 | 36.9 | 36.7 |
| | 2'' | 78.1 | 78.0 | 76.8 |
| | 3'' | 72.8 | 72.8 | 73.1 |
| | 4'' | 24.5 | 24.5 | 36.7 |
| | 2'''' | | | 80.4 |
| | 3'''' | | | 73.1 |
| | 4'''' | | | 21.8 |
| | 1 | 100.6 | 100.6 | 100.8 |
| | 2 | 71.7 | 71.6 | 71.5 |
| Allose | 3 | 72.1 | 72.1 | 71.9 |
| moiety | 4 | 68.3 | 68.3 | 68.1 |
| | 5 | 74.9 | 74.8 | 74.2 |
| | 6 | 62.7 | 62.7 | 62.4 |

*Epicatechin-(4β→8)-epicatechin-(4β→8)-catechin 3-O-β-D-allopyranoside* (**7**). A brown amorphous powder, $[\alpha]_D^{24} -182.0°$ ($Me_2CO$; *c* 0.69). Negative FABMS *m/z*: 1027 $[M-H]^-$. The $^1H$ NMR spectrum was complicated by rotational isomerism. (Found; C, 56.82; H, 5.14. $C_{51}H_{48}O_{23} \cdot 3H_2O$ requires: C, 56.56; H, 5.03%).

*Partial thiolytic degradation of* **7**. A mixture of **7** (84 mg), benzylmercaptan (3 ml) and HOAc (2 ml) in EtOH (10 ml) was refluxed for 2 hr with stirring. The concd reaction mixture was repeatedly chromatographed on Sephadex LH-20 (EtOH and 80% aq. MeOH) to yield (−)-epicatechin 4β-benzylthioether (23 mg), procyanidin B-2 4''β-benzylthioether (7 mg), (+)-catechin 3-*O*-β-D-allopyranoside (23 mg) and procyanidin B-1 3''-*O*-β-D-*allopyranoside* (12 mg) (**11**). Compound **11**: a brown amorphous powder, $[\alpha]_D^{26} + 127.7°$ ($Me_2CO$; *c* 0.57). Negative FABMS *m/z*: 739 $[M-H]^-$. $^1H$ NMR ($Me_2CO$-$d_6 + D_2O$): δ2.5–3.0 (2H, *m*, H-4''), 3.3–4.0 (5H, *m*, sugar-H), 4.00 (1H, *s*, H-3), 4.08 (1H, *br s*, allosyl H-3), 4.28 (1H, *m*, H-3''), 4.63 (1H, *s*, H-4), 4.91 (1H, *d*, $J = 8$ Hz, anomeric H), 5.20 (1H, *s*, H-2), 5.21 (1H, *d*, $J = 5$ Hz, H-2''), 5.9–6.1 (3H, *m*, A-ring H), 6.7–7.3 (6H, *m*, B-ring H).

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