Constituents of a Fern, *Davallia mariesii* MOORE. V.[1,2] Isolation and Structures of Davallin, a New Tetrameric Proanthocyanidin, and Two New Phenolic Glycosides

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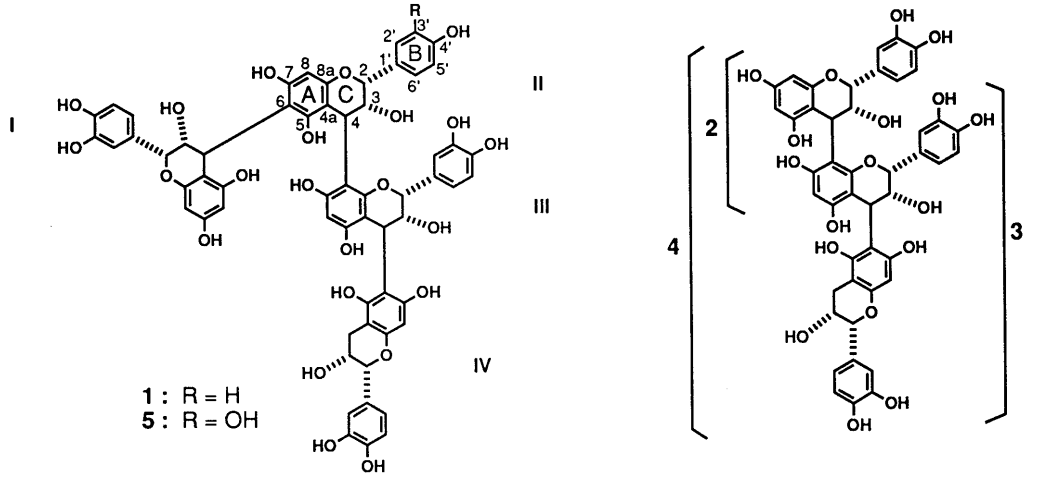
A new tetrameric proanthocyanidin named davallin (1) and two new phenolic glycosides, 5-*O*-β-D-(6-*O*-vanilloylglucopyranosyl)gentisic acid (6) and 4-*O*-β-D-(6-*O*-vanilloylglucopyranosyl)vanillic acid (7), have been isolated from the rhizomes of *Davallia mariesii* MOORE together with six known compounds, procyanidin B-2 (2) and B-5 (3), epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin (4), epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin (5), protocatechuic acid (8), and 1-naphthol-β-D-glucopyranoside (9). Structures of the three new compounds (1, 6, and 7) were determined by means of spectroscopic methods including two-dimensional NMR techniques.

Among the compounds obtained, 1 and 2 inhibited protein kinase C with IC_{50} values of 3.5 and 8.6 μM, respectively.

Keywords *Davallia mariesii*; davallin; protein kinase C inhibitor; condensed tannin; 5-*O*-β-D-(6-*O*-vanilloylglucopyranosyl)gentisic acid; 4-*O*-β-D-(6-*O*-vanilloylglucopyranosyl)vanillic acid

In previous papers,[1,3] we reported the isolation and structure elucidation of davallialactone, the 7-*O*-β-D-glucuronide of (±)-eriodictyol, davalliosides A and B, (−)-epicatechin-5-*O*-β-D-glucopyranoside, 5,7-dihydroxychromone-7-*O*-β-D-glucuronide methyl ester, marioside, and four proanthocyanidins from the rhizomes of *Davallia mariesii* MOORE. In a continuation of that work, we have isolated a new tetrameric proanthocyanidin named davallin (**1**) and two new phenolic glycosides, 5-*O*-β-D-(6-*O*-vanilloylglucopyranosyl)gentisic acid (**6**) and 4-*O*-β-D-(6-*O*-vanilloylglucopyranosyl)vanillic acid (**7**), together with six known compounds. This paper deals with the isolation and



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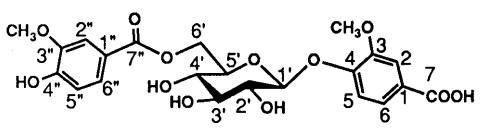

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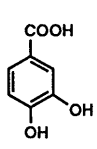

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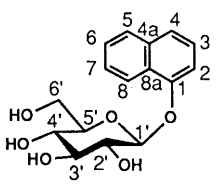

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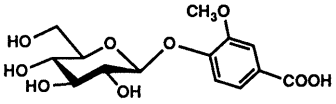

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Chart 1

structure elucidation of the three new compounds and the identification of the six known compounds.

An aqueous acetone extract of air-dried rhizomes of *D. mariesii* was fractionated according to the procedure in a previous paper[3a] to give an ethyl acetate-soluble fraction, which showed an inhibitory effect toward protein kinase C. This fraction was separated by a combination of silica gel and Sephadex LH-20 column chromatography and preparative TLC to give a new proanthocyanidin named davallin (**1**) and two new phenolic glycosides (**6** and **7**) together with six known compounds. Among the known compounds, five were identified as procyanidins B-2 (**2**)[4] and B-5 (**3**),[3c] epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin (**4**),[3c] and epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin (**5**),[3c] and protocatechuic acid (**8**)[3c] by direct comparison with authentic samples, while the other one (**9**) was determined to be 1-naphthol-β-D-glucopyranoside[5] by detailed analysis of its spectral data.

Davallin (**1**) was obtained as a pale brown amorphous powder and showed $[\alpha]_D^{22}$ +185.8° (MeOH). It showed a dark-blue color with ferric chloride reagent and an orange-red color with anisaldehyde–sulfuric acid reagent, and its UV and IR spectra were closely similar to those of **5**,[3c] suggesting that **1** is a condensed tannin. Elemental analysis data of **1** and the *quasi*-molecular ion peak at *m/z* 1139 $[M+H]^+$ in the fast atom bombardment MS (FAB-MS) were consistent with the molecular formula $C_{60}H_{50}O_{23}$, which is one oxygen atom less than that of **5**.

Acid-catalyzed degradation[6] of **1** with toluene-α-thiol afforded (−)-epicatechin (**10**),[3c] (−)-4β-benzylthioepicatechin (**11**),[3c] and (−)-4β-benzylthioepiafzelechin (**12**)[7a] in a molar ratio of 1:2:1 (Chart 2). Therefore, **1** was considered to be a tetrameric proanthocyanidin composed of a (−)-epiafzelechin and three (−)-epicatechin units, with the lower terminal unit being (−)-epicatechin.

The ^{1}H- and ^{13}C-NMR spectra of **1** in methanol-d_4 showed complicated patterns due to two conformers formed by rotational isomerism but they closely resembled those of **5**,[3c] and as in the case of **5**, the spectra in a mixture of methanol-d_4–D_2O (2:1) showed simple patterns which were ascribed to a single conformer.

Detailed analysis of the ^{1}H–^{1}H shift correlation spectrum (COSY) (Fig. 1) of **1** enabled us to correlate all protons in rings B/C in each flavanol unit with each other. For instance, correlation peaks between 2-H (δ 4.86), 3-H (δ 3.82), and

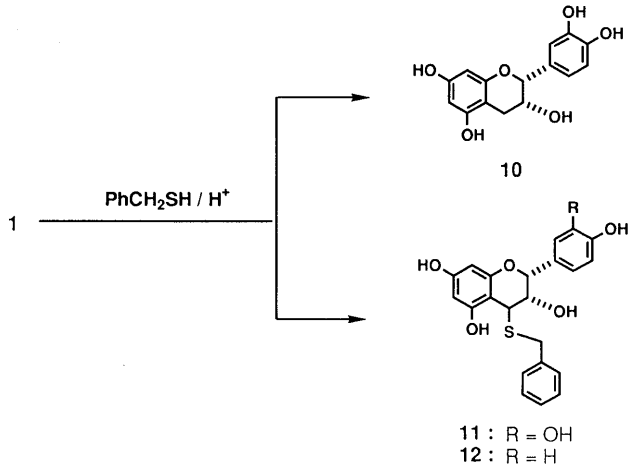

Chart 2

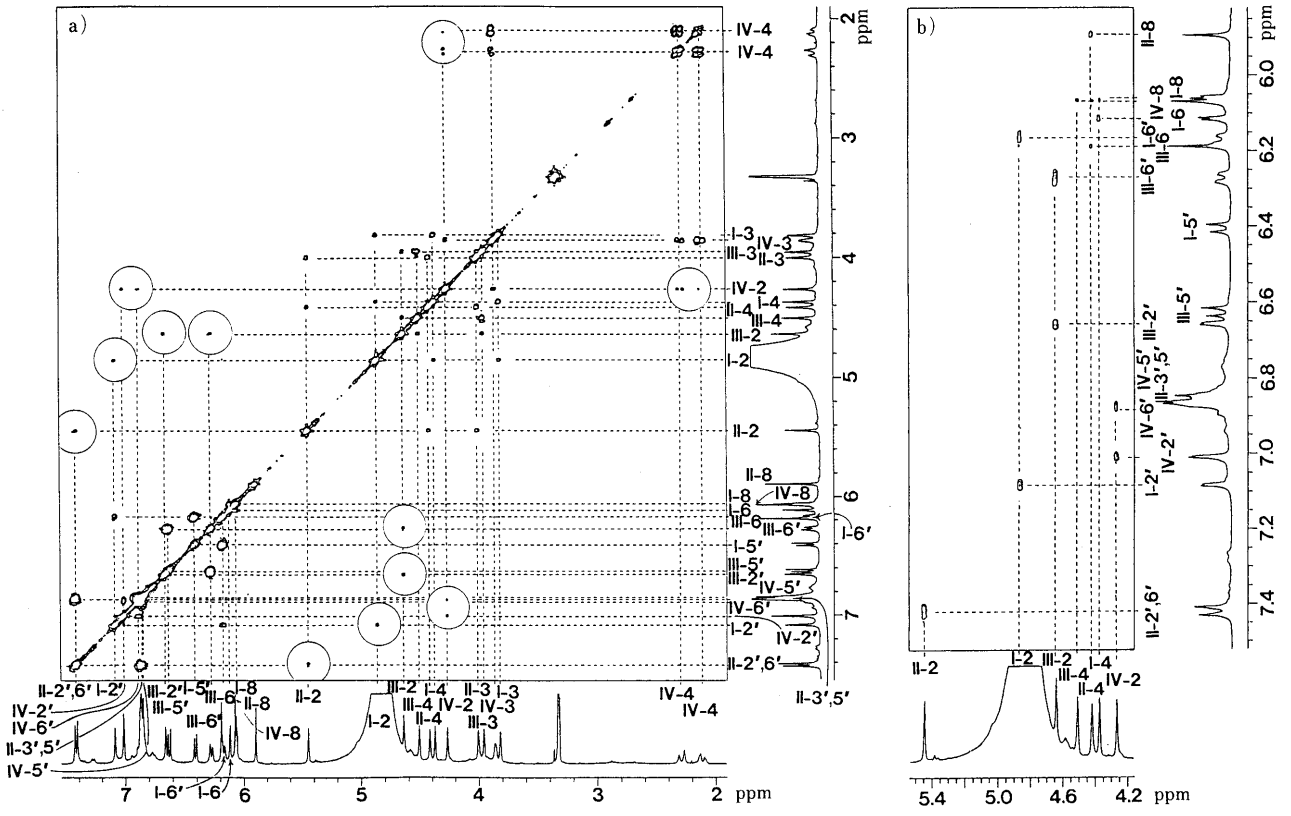

Fig. 1. ^{1}H–^{1}H COSY Spectrum of **1** in CD_3OD–D_2O (2:1)

a) Whole region. b) Cross peaks between ring C and rings A/B protons.

TABLE I. 400 MHz ^{1}H- and 100 MHz ^{13}C-NMR Data for **1** and **5** in CD_3OD–D_2O

Units	No.	**1**				**5**[a]	
		δ_H (J in Hz)	δ_C	^{1}H L.r. coupled ($^3J_{CH}$ $^2J_{CH}$)[b]		δ_H (J in Hz)	δ_C
I	2	4.86 br s	77.7 d			4.83 br s	77.78 d
	3	3.82 br s	73.2 d			3.79 br s	73.2 d
	4	4.37 br s	38.3 d			4.34 br s	38.3 d
	4a	—	99.9 s	I3, I6, I8	I4	—	100.0 s
	5	—	158.3 s	I4	I6	—	158.4 s
	6	6.11[c] d (2.1)	97.16 d	I8		6.10 d (2.4)	97.3 d
	7	—	159.4 s		I6, I8	—	159.5 s
	8	6.06[c] d (2.1)	97.6 d	I6		6.04 d (2.4)	97.7 d
	8a	—	159.6 s	I4	I8	—	159.7 s
	1′	—	133.2 s	I5′	I2	—	133.3 s
	2′	7.08[d] d (1.8)	116.4 d	I2, I6′		7.06[d] d (1.5)	116.5 d
	3′	—	145.94 s	I5′	I2′	—	145.9 s
	4′	—	145.82 s	I2′, I6′	I5′	—	145.8 s
	5′	6.41 d (8.2)	116.2 d		I6′	6.39 d (8.2)	116.3 d
	6′	6.18[d] dd (8.2, 1.8)	120.9 d	I2, I2′		6.14[d] dd (8.2, 1.5)	121.0 d
II	2	5.44 br s	77.4 d			5.36 br s	77.5 d
	3	4.00 br s	74.1 d			3.98 br s	74.2 d
	4	4.42 br s	38.6 d			4.38 br s	38.7 d
	4a	—	104.7 s	II3, II8	II4	—	104.9 s
	5	—	156.5 s	II4, I4		—	156.5 s
	6	—	106.8[e] s	II8, I3	I4	—	106.9 s
	7	—	155.1 s	I4	II8	—	155.1 s
	8	5.89[c] s	97.19 d			5.87 s	97.2 d
	8a	—	155.64 s	II4	II8	—	155.7 s
	1′	—	132.9 s	II3′, II5′	II2	—	133.6 s
	2′	7.42[d] d (8.9)	130.2 d	II2, II6′	II3′	7.05[d] d (1.8)	116.6 d
	3′	6.86 d (8.9)	116.6 d	II5′	II2′	—	145.9 s
	4′	—	157.5 s	II2′, II6′	II3′, II5′	—	145.98 s
	5′	6.86 d (8.9)	116.6 d	II3′	II6′	6.82 d (8.3)	117.1 d
	6′	7.42[d] d (8.9)	130.2 d	II2, II2′	II5′	6.90[d] dd (8.3, 1.8)	121.0 d
III	2	4.64 br s	77.8 d			4.61 br s	77.84 d
	3	3.96 d (1.8)	72.4 d			3.93 d (1.8)	72.5 d
	4	4.51 br s	38.5 d			4.48 br s	38.6 d
	4a	—	99.9 s	III3, III6	III4	—	100.0 s
	5	—	157.3 s	III4	III6	—	157.4 s
	6	6.19[c] s	97.8 d			6.17 s	97.8 d
	7	—	157.5 s	II4	III6	—	157.6 s
	8	—	110.1[e] s	III6, II3	II4	—	110.2 s
	8a	—	156.6 s	III4, II4		—	156.7 s
	1′	—	132.2 s	III5′	III2	—	132.3 s
	2′	6.66[d] d (1.8)	115.9 d	III2, III6′		6.63[d] d (1.8)	116.0 d
	3′	—	145.4 s	III5′	III2′	—	145.44 s
	4′	—	145.3 s	III2′, III6′	III5′	—	145.41 s
	5′	6.63 d (8.2)	116.5 d			6.61 d (8.3)	116.6 d
	6′	6.27[d] dd (8.2, 1.8)	120.9 d	III2, III2′		6.24[d] dd (8.3, 1.8)	121.0 d
IV	2	4.27 br s	80.0 d			4.24 br s	80.1 d
	3	3.86 br s	68.3 d			3.84 br s	68.3 d
	4	2.29 br d (16.7)	29.8 t			2.26 br d (16.9)	29.9 t
		2.11 dd (16.7, 4.4)				2.09 dd (16.9, 4.6)	
	4a	—	102.5 s	IV3, IV8	IV4	—	102.6 s
	5	—	156.4 s	IV4, III4		—	156.5 s
	6	—	108.3[e] s	IV8, III3	III4	—	108.4 s
	7	—	155.2 s	III4	IV8	—	155.3 s
	8	6.07[c] s	96.9 d			6.05 s	96.9 d
	8a	—	155.57 s	IV4	IV8	—	155.6 s
	1′	—	133.0 s	IV5′	IV2	—	133.1 s
	2′	7.01[d] d (1.5)	116.2 d	IV2, IV6′		6.99[d] d (1.5)	116.3 d
	3′	—	145.88 s	IV5′	IV2′	—	146.04 s
	4′	—	145.7 s	IV2′, IV6′	IV5′	—	146.04 s
	5′	6.85 d (8.2)	117.1 d			6.83 d (8.2)	117.1 d
	6′	6.89[d] dd (8.2, 1.5)	120.7 d	IV2, IV2′		6.86[d] dd (8.2, 1.5)	120.8 d

a) Signals were assigned by 2D NMR spectroscopy.[3c] *b*) $^2J_{CH}$ and $^3J_{CH}$ indicate the protons coupled with the carbon through two and three bonds, respectively, which were observed in the HMBC spectrum. *c, e*) Assignments were done by comparison with the data of **5**. *d*) Long-range ^{1}H–^{1}H coupling was observed with 2-H of each unit in the ^{1}H–^{1}H COSY.

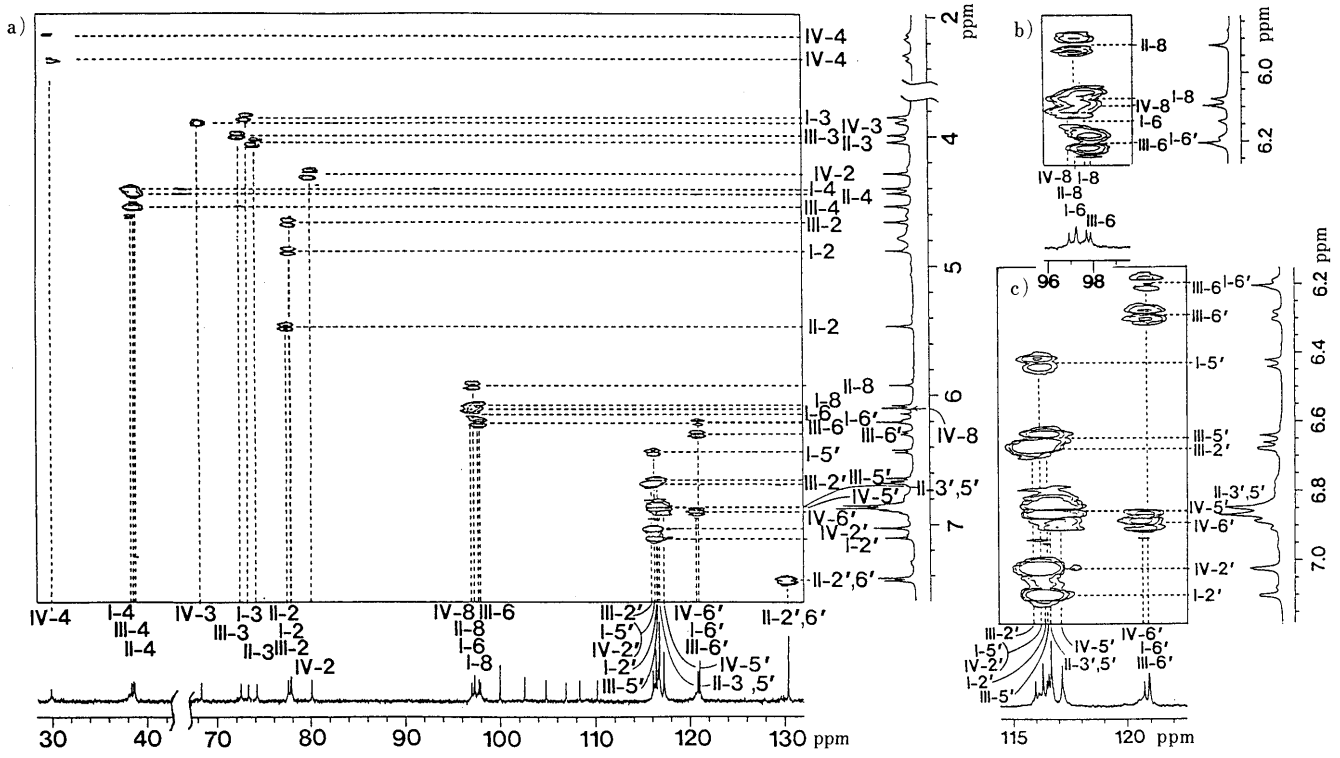

Fig. 2. HMQC Spectrum of **1** in CD_3OD–D_2O (2:1)

Sample, 123 mg; $^1J_{CH}$ = 135 Hz; 15 h run. a) Whole region. b) Cross peaks of ring A methine groups. c) Cross peaks of ring B methine groups.

4-H (δ 4.37) and between 2′-H (δ 7.08), 5′-H (δ 6.41), and 6′-H (δ 6.18) in an epicatechin unit were clearly recognized and long-range correlations between the proton 2-H and the protons 2′-H and 6′-H were also observed (Table I). Similarly, protons of rings B/C in the epiafzelechin unit were discriminated from those in the other units. Also, the rings B/C protons of the lower terminal epicatechin unit (unit IV) were readily assigned by tracing the ^{1}H–^{1}H correlation peaks starting from the characteristic AB-type signals due to 4-H_2 (δ 2.11, 2.29). Furthermore, as can be seen in Fig. 1b, 4-H in unit I (δ 4.37) showed weak but significant long-range correlation peaks through five bonds[8] with the *meta*-coupled A-ring protons at δ 6.06 and 6.11 (each d, J = 2.1 Hz, 8-H and 6-H in unit I), suggesting that the upper terminal unit (unit I) in **1** is an epicatechin unit. Thus the epiafzelechin unit must form either the second or the third upper terminal unit.

^{13}C-Signals of the methine and methylene carbons in each flavanol unit were readily assigned by careful analysis of the ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectrum[9] (Fig. 2 and Table I).

We then measured the ^{1}H-detected hereronuclear multiple bond connectivity (HMBC) spectrum[9b,10] of **1** in order to clarify the connectivity of the flavanol units. As can be seen in Fig. 3, the quaternary carbon signal at δ 99.9 (2 × C, C-I-4a, C-III-4a) shows long-range correlations with 3-H (δ 3.82), 4-H (δ 4.37), and both the *meta*-coupled benzene protons (δ 6.06, 6.11) of the upper terminal unit (unit I), suggesting that one of the carbons at δ 99.9 can be assigned to C-4a of unit I. Both 3-H (δ 3.82) and 4-H (δ 4.37) of unit I further show long-range correlations with the quaternary carbon at δ 106.8 (C-II-6), which is also correlated with the benzene proton at δ 5.89 (H-II-8). Thus, it is reasonable to suppose that the carbon (δ 106.8) is the ring-A carbon of the second upper terminal unit (unit II) connecting with C-4 of the upper terminal unit (unit I), while the proton (δ 5.89) is the ring-A proton of the second upper terminal unit (unit II). Because the latter proton (δ 5.89) is correlated with the quaternary carbon at δ 104.7 (C-II-4a) which, in turn, shows long-range correlations with both 3-H (δ 4.00) and 4-H (δ 4.42) of the epiafzelechin unit, the carbon at δ 104.7 is assigned to C-4a of the epiafzelechin unit and the epiafzelechin is concluded to form the second upper terminal unit (unit II) in **1**. Thus, the third unit (unit III) must be an epicatechin unit.

The locations and relative stereochemistry of interflavonoid linkages in **1** were concluded to be the same as those of **5** in view of the apparent close similarity of the ^{1}H- and ^{13}C-NMR spectra (Table I).

Based on these findings, davallin was determined to be epicatechin-(4β→6)-epiafzelechin-(4β→8)-epicatechin-(4β→6)-epicatechin (**1**).

Compound **6** was obtained as an off-white amorphous solid, $[\alpha]_D^{22}$ −63.4° (MeOH), and showed *quasi*-molecular ion peaks at *m/z* 467 $[M+H]^+$ and 489 $[M+Na]^+$ in the FAB-MS. In the UV spectrum, **6** showed absorption bands at 224 (log ε 4.33), 263 (4.06), and 297 nm (3.95) and in the IR spectrum it showed absorptions at 3428 (OH), 3250—2400 (br, COOH), 1701 (conj. COOR), 1689 (conj. COOH), 1638, 1599, and 1517 cm^{-1} (aromatic ring). The ^{1}H- and ^{13}C-NMR spectra of **6**, analyzed with the aid of ^{1}H–^{1}H and ^{1}H–^{13}C COSY, revealed the presence of two 1,3,4-trisubstituted benzene rings, a methoxyl group, and a glucose residue (Table II). Also, the ^{13}C-NMR spectrum of **6** showed two signals due to carbonyl carbons at δ 168.8 and 176.0, indicating that **6** may be a glucoside of a benzoic

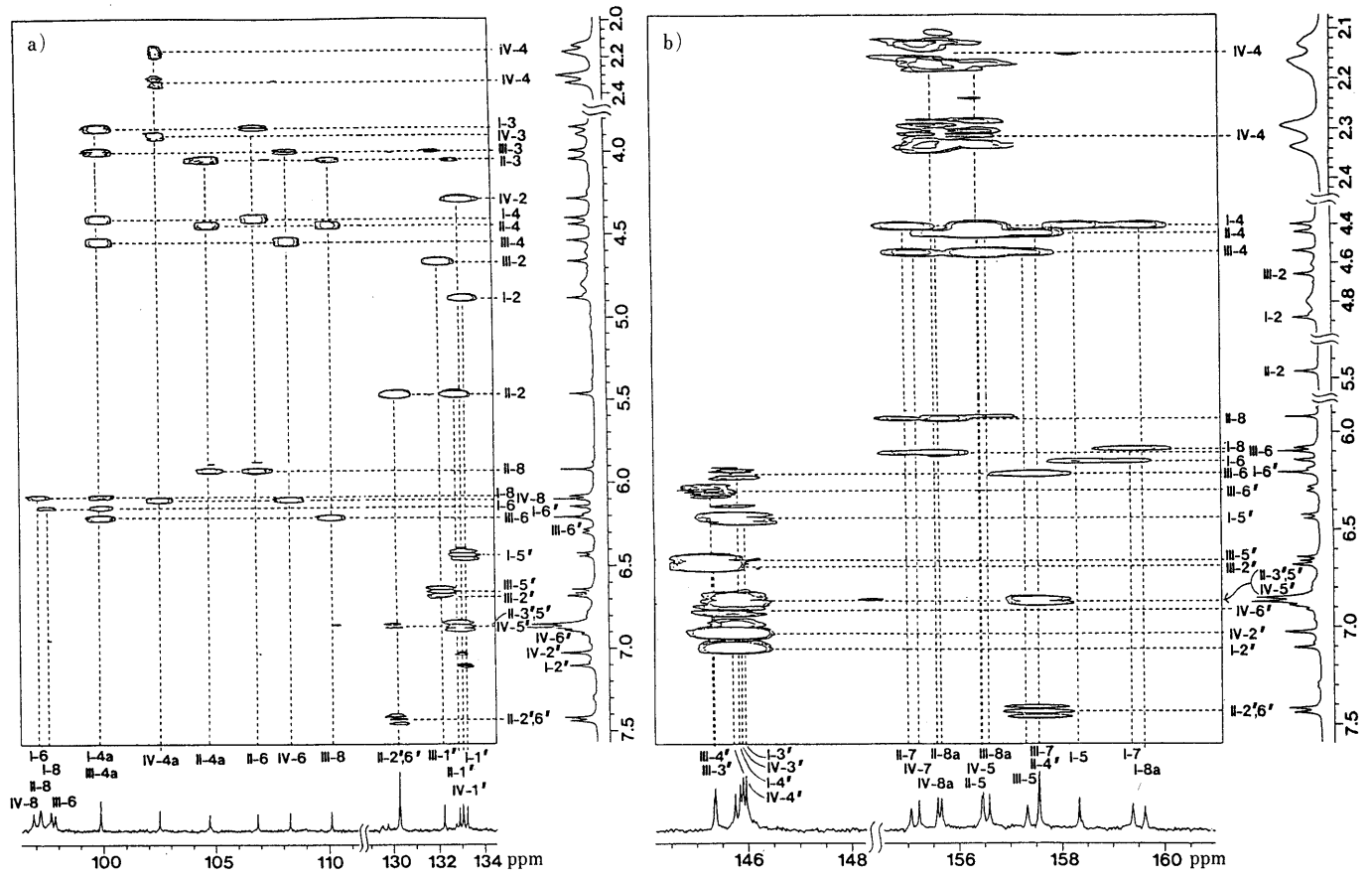

Fig. 3. HMBC Spectrum of **1** in CD_3OD–D_2O (2:1)

Sample, 123 mg; $^{1r}J_{CH}$ = 6 Hz; 108 h run. a) High-field region. b) Low-field region.

TABLE II. 400 MHz ^{1}H- and 100 MHz ^{13}C-NMR Data for **6**, **7**, and **13** in Methanol-d_4 (Coupling Constants in Hz in Parenthesis)

Position	**6**[a]		**7**[b]		**13**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	—	120.2 s	—	127.9 s	—	126.8 s
2	—	158.9 s	7.59 d (1.8)	115.2 d	7.60 d (1.8)	115.2 d
3	6.61 d (8.9)	118.4 d	—	151.2 s	—	151.1 s
4	7.10 dd (8.9, 3.1)	124.9 d	—	152.2 s	—	152.8 s
5	—	151.4 s	7.12 d (8.4)	117.2 d	7.20 d (8.2)	117.2 d
6	7.59 d (3.1)	120.6 d	7.43 dd (8.4, 1.8)	125.3 d	7.63 dd (8.2, 1.8)	125.6 d
7	—	176.0 s	—	170.9 s	—	170.3 s
3-OCH_3	—	—	3.88[c]	57.4 q	3.89[c] s	57.5 q
1′	4.84 d (7.6)	104.3 d	5.04 d (7.3)	102.6 d	5.02 d (7.3)	102.7 d
2′	3.48 dd (8.5, 7.6)	75.7 d	3.57 dd (8.5, 7.3)	75.5 d	3.54 dd (9.2, 7.3)	75.5 d
3′	3.53 t (8.5)	78.6 d	3.52 t (8.5)	78.5 d	3.49 dd (9.2, 8.2)	78.6 d
4′	3.46 dd (8.9, 8.5)	72.7 d	3.43 dd (9.7, 8.5)	72.7 d	3.41 dd (9.8, 8.2)	72.0 d
5′	3.75 ddd (8.9, 6.7, 2.1)	76.2 d	3.81 ddd (9.7, 7.6, 2.1)	76.6 d	3.47 ddd (9.8, 5.5, 2.1)	79.2 d
6′	4.39 dd (11.9, 6.7)	65.9 t	3.55 dd (11.9, 7.6)	65.8 t	3.70 dd (12.2, 5.5)	63.2 t
	4.68 dd (11.9, 2.1)		4.70 dd (11.9, 2.1)		3.88 dd (12.2, 2.1)	
1″	—	123.2 s	—	123.1 s		
2″	7.52 d (1.8)	114.5 d	7.51 d (1.8)	114.5 d		
3″	—	149.5 s	—	149.6 s		
4″	—	153.6 s	—	153.8 s		
5″	6.87 d (8.2)	116.8 d	6.86 d (8.2)	116.8 d		
6″	7.57 dd (8.2, 1.8)	126.1 d	7.56 dd (8.2, 1.8)	126.0 d		
7″	—	168.8 s	—	168.6 s		
3″-OCH_3	3.85[d] s	57.3 q	3.85[d]	57.3 q		

a) Assignments are based on the results of ^{1}H–^{1}H and ^{1}H–^{13}C COSY and HMBC spectrum. *b*) Assignments are based on a comparison with the data of **6** and **13**. *c*, *d*) Long-range ^{1}H–^{1}H correlations and NOE's were observed with 2-H and 2″-H, respectively.

acid derivative.

In the HMBC spectrum of **6**, the carbonyl carbon at δ 168.8 (C-7″) showed long-range correlations with the aromatic protons at δ 7.52 (d, J=1.8 Hz, 2″-H) and 7.57 (dd, J=8.2, 1.8 Hz, 6″-H). The oxygenated sp^2 quaternary carbon at δ 153.6 (C-4″) showed long-range correlations with the proton at δ 6.87 (d, J=8.2 Hz, 5″-H) and with 2″-H and 6″-H, while another oxygenated quaternary carbon at δ 149.5 (C-3″) was correlated with 2″-H, 5″-H, and the methoxy protons, suggesting the presence of a vanillic acid residue in **6**. Likewise, the presence of a gentisic acid residue was established based on the long-range correlations between the carbonyl carbon at δ 176.0 (C-7) and 6-H and between the oxygenated sp^2 quaternary carbons (C-2 and C-5) and three aromatic protons (3-H, 4-H, and 6-H).

Because the carbon C-7″ showed long-range correlations with 6′-H_2 of the glucose residue, the vanillic acid residue must be located at the C-6′ position of the glucose residue. On the other hand, the location of the glucoside linkage was determined to be at the C-5 position of the gentisic acid residue based on the result of difference nuclear Overhauser effect (NOE) experiments between the anomeric proton and 4-H and 6-H.

From the above evidence, **6** was determined to be 5-*O*-*β*-D-(6-*O*-vanilloylglucopyranosyl)gentisic acid (**6**).

Compound **7**, an off-white amorphous solid, $[\alpha]_D^{22}$ −82.3° (MeOH), showed UV and IR absorptions similar to those of **6**, and revealed *quasi*-molecular ion peaks at m/z 481 $[M+H]^+$ and 503 $[M+Na]^+$ in the FAB-MS.

The ^{1}H and ^{13}C-NMR spectra of **7** showed signal patterns, which were almost identical with those of the 6-*O*-vanilloylglucopyranose moiety in **6** and also with those of 4-*O*-*β*-D-glucopyranosylvanillic acid (**13**)[1] (Table II). Thus **7** was considered to be 4-*O*-*β*-D-(6-*O*-vanilloylglucopyranosyl)vanillic acid. This was confirmed by difference NOE experiments, where irradiation of the methoxy protons at δ 3.85 (3″-OC$\underline{H}_3$), and at δ 3.88 (3-OC$\underline{H}_3$) increased the intensities of 2″-H (δ 7.51) and of 2-H (δ 7.59), respectively, while irradiation of the anomeric proton (δ 5.04) increased the intensity of 5-H (δ 7.12).

From these findings, **7** was concluded to be 4-*O*-*β*-D-(6-*O*-vanilloylglucopyranosyl)vanillic acid (**7**).

This work has provided three new compounds, davallin (**1**), 5-*O*-*β*-D-(6-*O*-vanilloylglucopyranosyl)gentisic acid (**6**), and 4-*O*-*β*-D-(6-*O*-vanilloylglucopyranosyl)vanillic acid (**7**). Even though many olygomeric proanthocyanidins have hitherto been reported, only a few dimeric proanthocyanidins composed of epicatechin and epiafzelechin are known,[7] and davallin (**1**) is the first example of a tetrameric proanthocyanidin of this novel class. On the other hand, 1-naphthol-*β*-D-glucopyranoside (**9**) has been isolated for the first time from a natural source.[5]

Like the proanthocyanidins **3**, **4**, and **5**,[3c] **1** and **2** showed an inhibitory effect toward protein kinase C with IC_{50} values of 3.5 and 8.6 μM, respectively.[11]

Experimental

Optical rotations were measured on a JASCO DIP-4 automatic polarimeter. UV spectra were taken with a Shimadzu 202 UV spectrophotometer in MeOH solutions and IR spectra on a JASCO IR-2 spectrometer in KBr discs. FAB-MS were obtained with a JEOL D-300 or JMS-SX102 mass spectrometer using a direct inlet system and glycerol was used as a matrix unless otherwise noted. ^{1}H-, ^{13}C-, and two dimensional (2D) NMR spectra and difference NOE spectra were taken with a JEOL JNM-GX400 spectrometer with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ values. Multiplicities of ^{13}C-NMR signals were determined by means of the distortionless enhancement by polarization transfer (DEPT) method and are indicated as s (singlet), d (doublet), t (triplet), and q (quartet).

Column chromatography was done with Sephadex LH-20 (Pharmacia) or silica gel (Mallinckrodt, 100 mesh). TLC and preparative TLC were carried out on precoated Merck Kieselgel 60 F_{254} plates (0.25 or 0.5 mm), and spots were detected under UV light or by using $FeCl_3$, anisaldehyde–H_2SO_4, or $Ce(SO_4)_2$–10%H_2SO_4 (1:99) reagents.

Extraction, Fractionation, and Separation Air-dried rhizomes (17 kg) of *Davallia mariesii* MOORE, collected at Pusan, South Korea, in 1989, were extracted and fractionated according to the procedure in a previous paper[3a] to give an EtOAc-soluble fraction (106 g). This was subjected to column chromatography on Sephadex LH-20 (bed, 5×54 cm) and eluted successively with EtOH and EtOH–MeOH (50:50). The eluates were collected in 220 ml portions, monitored by TLC, and finally combined into thirteen fractions [fractions 1 to 9, EtOH eluate; fractions 10 to 13, EtOH–MeOH (50:50) eluate].

Fraction 2 (2.5 g) was chromatographed on a silica gel column (50 g) with $CHCl_3$–MeOH (90:10—60:40). The eluates were collected in 18 ml portions, monitored by TLC, and combined into seven fractions (fraction 2-1 to 2-7). Fraction 2-2 [$CHCl_3$–MeOH (90:10) eluate, 385 mg] was again separated by column chromatography on Sephadex LH-20 with EtOH–H_2O (50:50) to give seven fractions (fraction 2-2-1 to 2-2-7). Fractions 2-2-2 (27 mg) and 2-2-4 (190 mg) were separated by preparative TLC with EtOAc–EtOH–H_2O (16:2:1), and the former gave 4-*O*-*β*-D-(6-*O*-vanilloylglucopyranosyl)vanillic acid (**7**, 1.6 mg) and the latter gave protocatechuic acid (**8**, 34 mg) from a less polar zone and 1-naphthol-*β*-D-glucopyranoside (**9**, 4.0 mg)[5] from a more polar zone. On the other hand, fraction 2-3 [$CHCl_3$–MeOH (90:10) eluate, 638 mg] was subjected to preparative TLC with EtOAc–EtOH–H_2O (16:2:1), and protocatechuic acid (**8**, 24 mg) was obtained from a less polar fraction and 5-*O*-*β*-D-(6-*O*-vanilloylglucopyranosyl)gentisic acid (**6**, 98 mg) from a more polar fraction.

Fraction 4 (3 g) was separated by column chromatography on Sephadex LH-20 (bed, 3.5×36 cm) with EtOH to furnish procyanidin B-2 (**2**, 1.3 g).[4]

Fractions 6 (1.2 g), 8 (1.3 g), and 12 (3.8 g) afforded procyanidin B-5 (**3**), epicatechin-(4*β*→8)-epicatechin-(4*β*→6)-epicatechin (**4**), and epicatechin-(4*β*→6)-epicatechin-(4*β*→8)-epicatechin-(4*β*→6)-epicatechin (**5**), respectively.[3c]

Fraction 10 (3.9 g) was again subjected to column chromatography over Sephadex LH-20 using MeOH–H_2O with increasing amounts of MeOH (0:100—70:30) and a fraction eluted with MeOH–H_2O (60:40) gave davallin (**1**, 995 mg).

Davallin (1) A pale brown amorphous solid, $[\alpha]_D^{22}$ +185.8° (c=0.67, MeOH). UV λ_{max} nm (log ε): 223 (4.84), 281 (4.18). IR ν_{max} cm^{-1}: 3350 (br, OH), 1600, 1510, 1440 (aromatic ring). Positive ion FAB-MS [matrix, glycerol–thioglycerol (1:1)] m/z: 1139 $[M+H]^+$. *Anal.* Calcd for $C_{60}H_{50}O_{23}\cdot 2H_2O$: C, 60.40; H, 4.73. Found: C, 60.31; H, 4.63. ^{1}H- and ^{13}C-NMR: Table I.

Complete Thiolytic Degradation of Davallin (1) A solution of **1** (61 mg), toluene-*α*-thiol (1.5 ml), and acetic acid (2 ml) in EtOH (10 ml) was refluxed for 24 h with stirring. The reaction mixture was concentrated under reduced pressure and the residue was chromatographed over Sephadex LH-20 (bed, 1.3×7 cm). After removal of toluene-*α*-thiol by elution with CH_2Cl_2, a mixture of products was eluted with MeOH. This mixture was subjected to preparative TLC with $CHCl_3$–MeOH (80:20) and separated into three fractions. The most polar fraction gave (−)-epicatechin (**10**, 11.8 mg)[3c] as a pale brown amorphous solid, $[\alpha]_D^{24}$ −54.2° (c=1.5, MeOH), and the next most polar fraction afforded (−)-4*β*-benzylthioepicatechin (**11**, 35.4 mg)[3c] as a pale brown amorphous powder, $[\alpha]_D^{24}$ −30.2° (c=1.5, MeOH). On the other hand, the less polar fraction gave (−)-4*β*-benzylthioepiafzelechin (**12**, 13 mg)[7] as a pale brown amorphous powder, $[\alpha]_D^{22}$ −35.8° (c=1.5, MeOH). Positive ion FAB-MS m/z: 397 $[M+H]^+$. ^{1}H-NMR (methanol-d_4) δ: 7.41 (2H, dd, J=7.3, 2.0 Hz, 2″,6″-H), 7.29 (2H, t, J=7.3 Hz, 3″,5″-H), 7.22 (1H, tt, J=7.3, 2.0 Hz, 4″-H), 7.19 (2H, d, J=8.5 Hz, 2′,6′-H), 6.76 (2H, d, J=8.5 Hz, 3′,5′-H), 5.96 (1H, d, J=2.3 Hz, 6-H), 5.89 (1H, d, J=2.3 Hz, 8-H), 5.27 (1H, br s, 2-H), 4.06 (1H, d, J=2.3 Hz, 4-H), 3.96 (2H, s, –SC$\underline{H}_2$–), 3.83 (1H, dd, J=2.3, 1.0 Hz, 3-H). ^{13}C-NMR (methanol-d_4) δ: 159.9 (s, C-7), 159.7 (s, C-5), 158.7 (s, C-4′), 158.1 (s, C-8a), 141.5 (s, C-1″), 132.1 (s, C-1′), 130.8 (2×C, d, C-2″, 6″), 130.3 (2×C, d, C-3″,5″), 129.8 (2×C, d, C-2′,6′), 128.7 (d, C-4″), 116.6 (2×C, d, C-3′,5′), 101.0 (s, C-4a), 97.7 (d, C-6), 96.6 (d, C-8), 76.4

(d, C-2), 72.4 (d, C-3), 44.9 (d, C-4), 38.8 (t, –SC$\underline{H}_2$–). These ^{1}H- and ^{13}C-signals were analyzed by the use of ^{1}H–^{1}H and ^{1}H–^{13}C COSY and HMBC spectra.

5-*O*-*β*-D-(6-*O*-Vanilloylglucopyranosyl)gentisic Acid (6) An off-white amorphous solid, $[\alpha]_D^{22}$ −63.4° (c=0.5, MeOH). UV λ_{max} nm (log ε): 224 (4.33), 263 (4.06), 297 (3.95). IR ν_{max} cm^{-1}: 3428 (OH), 3250—2400 (br, COOH), 1701 (conj. COOR), 1689 (conj. COOH), 1638, 1599, 1517 (aromatic ring), 1286, 1069. Positive ion FAB-MS m/z: 467 $[M+H]^+$, 489 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Table II.

4-*O*-*β*-D-(6-*O*-Vanilloylglucopyranosyl)vanillic Acid (7) An off-white amorphous solid, $[\alpha]_D^{22}$ −82.3° (c=0.6, MeOH). UV λ_{max} nm (log ε): 256 (4.19), 290 (3.99). IR ν_{max} cm^{-1}: 3450 (OH), 3400—2400 (br, COOH), 1710 (conj. COOR), 1699 (conj. COOH), 1602, 1515, 1464 (aromatic ring), 1280, 1217, 1070. Positive ion FAB-MS m/z: 481 $[M+H]^+$, 503 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Table II.

1-Naphthol-*β*-D-glucopyranoside (9) An off-white amorphous solid, $[\alpha]_D^{22}$ −95.5° (c=0.67, MeOH). UV λ_{max} nm (log ε): 319.5 (3.28), 312 sh (3.33), 305 sh (3.56), 293 sh (3.85), 288 (3.88), 282 sh (3.87), 227 (4.42). IR ν_{max} cm^{-1}: 3350 (OH), 1600, 1580, 1505 (aromatic ring), 1400, 1265, 1240, 1080, 770, 740. Positive ion FAB-MS m/z: 329 $[M+Na]^+$, 144 [naphthol]$^+$. Negative ion FAB-MS m/z: 305 $[M-H]^-$, 143 [naphthol−H]$^-$. ^{1}H-NMR (methanol-d_4) δ: 8.38 (1H, m, 8-H), 7.80 (1H, m, 5-H), 7.51 (1H, d, J=8.2 Hz, 4-H), 7.46 (1H, m, 6-H), 7.45 (1H, m, 7-H), 7.38 (1H, dd, J=8.2, 7.6 Hz, 3-H), 7.21 (1H, dd, J=7.6, 0.9 Hz, 2-H), 5.10 (1H, d, J=7.8 Hz, 1′-H), 3.91 (1H, dd, J=12.2, 2.1 Hz, 6′-H), 3.72 (1H, dd, J=12.2, 5.2 Hz, 6′-H), 3.65 (1H, dd, J=8.9, 7.8 Hz, 2′-H), 3.52 (1H, t, J=8.9 Hz, 3′-H), 3.49 (1H, ddd, J=8.9, 5.2, 2.1 Hz, 5′-H), 3.44 (1H, t, J=8.9 Hz, 4′-H). ^{13}C-NMR (methanol-d_4) δ: 155.4 (s, C-1), 136.8 (s, C-4a), 129.2 (d, C-5), 128.1 (d, C-7), 127.7 (d, C-3), 127.1 (d, C-6), 124.1 (d, C-8), 123.8 (2×C, d, C-4 and s, C-4a), 111.2 (d, C-2), 103.4 (d, C-1′), 79.1 (d, C-5′), 79.0 (d, C-3′), 75.9 (d, C-2′), 72.2 (d, C-4′), 63.4 (t, C-6′). These ^{1}H- and ^{13}C-signals were analyzed by the use of ^{1}H–^{1}H COSY, HMQC, and HMBC spectra.

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