Constituents of a Fern, *Davallia mariesii* MOORE. IV.[1] Isolation and Structures of a Novel Norcarotane Sesquiterpene Glycoside, a Chromone Glucuronide, and Two Epicatechin Glycosides

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Three new compounds, (−)-epicatechin-5-*O*-β-D-glucopyranoside (1), 5,7-dihydroxychromone-7-*O*-β-D-glucuronide methyl ester (6), and a novel norcarotane sesquiterpene glucoside named marioside (7), have been isolated from the rhizomes of *Davallia mariesii* MOORE together with five known compounds, (−)-epicatechin-3-*O*-β-D-allopyranoside (2), coumaric acid-4-*O*-β-D-glucopyranoside (3), caffeic acid-4-*O*-β-D-glucopyranoside (4), vanillic acid-4-*O*-β-D-glucopyranoside (5), and L-tryptophan (8). The structures of the new compounds (1, 6, and 7) were determined by means of spectroscopic methods including two-dimensional nuclear magnetic resonance techniques.

Keywords *Davallia mariesii*; Davalliaceae; norcarotane sesquiterpene glucoside; marioside; (−)-epicatechin-5-*O*-β-D-glucopyranoside: 5,7-dihydroxychromone-7-*O*-β-D-glucuronide methyl ester; (−)-epicatechin-3-*O*-β-D-allopyranoside; ^{1}H-NMR; ^{13}C-NMR; HMBC

In previous papers,[1,2] we reported the isolation and structure elucidation of davallialactone, the 7-*O*-β-D-glucuronide of (±)-eriodictyol, davalliosides A and B, and four proanthocyanidins from the rhizomes of *Davallia mariesii* MOORE. In a continuing study, we have isolated three new compounds, (−)-epicatechin-5-*O*-β-D-glucopyranoside (**1**), 5,7-dihydroxychromone-7-*O*-β-D-glucuronide methyl ester (**6**), and a novel norcarotane sesquiterpene glucoside named marioside (**7**), together with five known compounds, (−)-epicatechin-3-*O*-β-D-allopyranoside (**2**),[3] coumaric acid-4-*O*-β-D-glucopyranoside (**3**),[2b] caffeic acid-4-*O*-β-D-glucopyranoside (**4**),[2b] vanillic acid-4-*O*-β-D-glucopyranoside (**5**),[4] and L-tryptophan (**8**). This paper describes the isolation and structure elucidation of the three new compounds and the identification of five known compounds.

The butanol-soluble fraction (DA-4)[2b] of the aqueous acetone extract from the rhizomes of *D. mariesii* was separated by a combination of silica gel and Sephadex LH-20 column chromatography and preparative thin-layer chromatography (preparative TLC) or high-performance liquid chromatography (HPLC) to give compounds **1** to **8** together with davalliosides A and B (**9** and **10**).[2a] Among them, compounds **2** and **5** were identified as (−)-epicatechin-3-*O*-β-D-allopyranoside (**2**)[3] (Table I) and vanillic acid 4-*O*-β-D-glucopyranoside (**5**)[4] by comparison of the spectral data with published values. On the other hand, **3**, **4**, and **8** were identified as coumaric acid-4-*O*-β-D-glucopyranoside (**3**),[2b] caffeic acid-4-*O*-β-D-glucopyranoside (**4**),[2b] and L-tryptophan (**8**) by direct comparison with authentic samples.

Compound **1** was obtained as colorless needles (MeOH) having double melting points, mp 196—198 °C and 240—241.5 °C, and showed $[\alpha]_D^{24}$ −30.6° (MeOH). The molecular formula of **1** was determined to be $C_{21}H_{24}O_{11}$ by elemental analysis and fast atom bombardment mass spectral (FAB-MS) measurement (*m/z*, 453 $[M+H]^+$). It showed a dark-blue color with ferric chloride reagent and an orange-red color with anisaldehyde–sulfuric acid reagent, and its ultraviolet (UV) and infrared (IR) spectra were very similar to those of (−)-epicatechin-3-*O*-β-D-allopyranoside (**2**) (see Experimental).

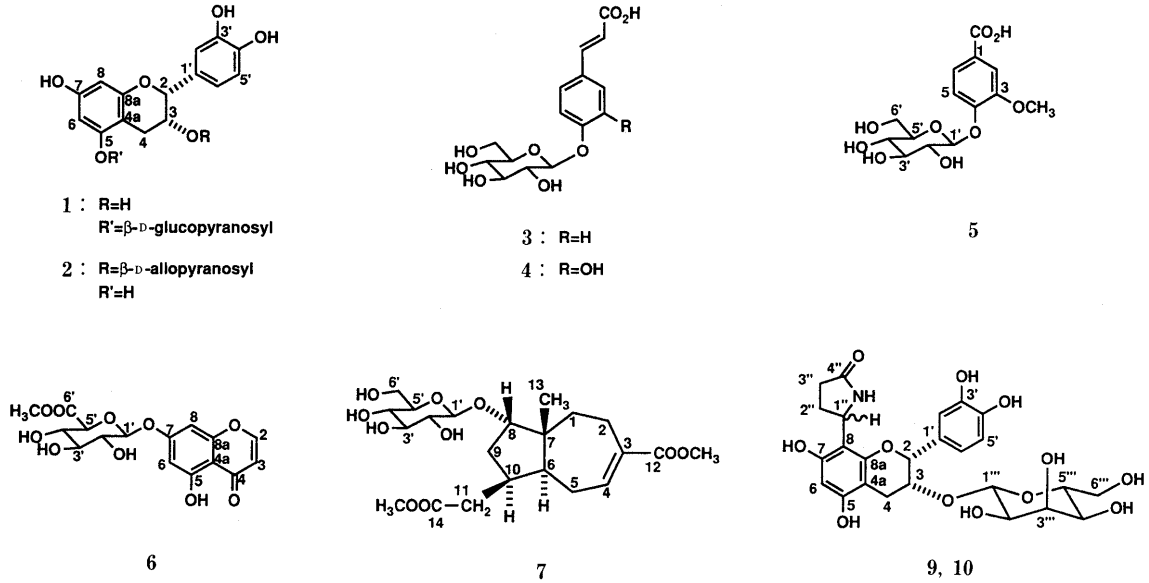

Chart 1

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** (dimethyl sulfoxide-d_6), analyzed with the aid of ^{1}H–^{1}H shift correlation spectroscopy (COSY), showed signals due to two methines (δ 4.74, br s, 2-H; δ 4.01, dt d, J=4.9, 3.4, 1.2 Hz, 3-H), a methylene (δ 2.71, 2H, d, J=3.4 Hz, 4-H_2), a pair of *meta*-coupled aromatic methines (δ 5.92 and 6.13, each d, J=2.1 Hz, 8-H and 6-H, respectively), and three coupled aromatic methines (δ 6.66—6.90), suggesting the presence of an epicatechin unit (Table I). The ^{13}C-NMR spectrum of **1** also showed ^{13}C-signals corresponding to the above groups (Table I). Moreover, the ^{1}H-NMR spectrum showed signals due to an anomeric proton at δ 4.72 (d, J=7.3 Hz) and four overlapping signals at δ 3.30—3.15, ascribable to methine protons of a sugar group. In pyridine-d_5, these signals were clearly separated and could be readily analyzed, leading to the conclusion that the sugar moiety is a β-glucopyranosyl group.

The position of the glucoside linkage in **1** was determined from the ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum (in dimethyl sulfoxide-d_6),[5] in which the anomeric proton (δ 4.72) showed a long-range correlation with the oxygenated quaternary carbon at δ 156.4, assignable to C-5 on the basis of the long-range correlations with 4-H_2 (δ 2.71, 2H) and 6-H (δ 6.13, see Table I). On the other hand, the ^{1}H-signal at δ 9.14 due to a hydroxyl proton showed a long-range correlation with the quaternary carbon at δ 155.4, which was ascribed to C-7 based on the long-range correlations with 6-H and 8-H (δ 6.13 and 5.92, respectively, Table I). It followed that the glucopyranosyl group must be located at C-5 and a hydroxyl group at C-7.

TABLE I. ^{1}H- and ^{13}C-NMR Data for **1** and **2** in Dimethyl Sulfoxide-d_6

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2	4.74 br s	78.2 d	5.14 d (3.1)	76.7 d
3	4.01 dtd (4.9, 3.4, 1.2)	64.7 d	4.23 ddd (7.9, 4.8, 3.1)	72.4 d
4	2.71[a] d (2H, 3.4)	28.2 t	2.34 dd (16, 7.9) 2.69[a] dd (16, 4.8)	23.0 t
4a	—	100.9 s	—	98.5[b] s
5	—	156.4 s	—	156.2 s
6	6.13[c] d (2.1)	96.5 d	5.89[c] d (2.2)	94.0 d
7	—	155.4 s	—	156.6 s
8	5.92[d] d (2.1)	95.2 d	5.75[d] d (2.2)	95.2 d
8a	—	156.8[e] s	—	155.1[e] s
1′	—	130.5 s	—	129.6 s
2′	6.90[a] br s	114.9 d	6.88[a] d (1.8)	115.3[f] d
3′	—	144.54 s	—	144.2 s
4′	—	144.48 s	—	144.4 s
5′	*ca.* 6.67	114.8 d	6.61 d (8.2)	114.7[f] d
6′	*ca.* 6.66	117.9 d	6.90[a] dd (8.2, 1.8)	118.6 d
1″	4.72 d (7.3)	100.7 d	4.59 d (7.5)	99.6[b] d
2″	3.30—3.15[g]	73.3 d	3.12 td (7.5, 3)	70.6 d
3″	3.30—3.15[g]	77.0 d	3.80 q (3)	71.6 d
4″	3.30—3.15[g]	69.6 d	3.26 ddd (9.5, 7.5, 3)	67.7 d
5″	3.30—3.15[g]	76.7 d	3.50 ddd (9.5, 6.1, 1.8)	74.4 d
6″	3.70 ddd (11.7, 5.7, 2) 3.51 dt (11.7, 5.7)	60.7 t	3.65 ddd (11.3, 5.7, 1.8) 3.40[h]	61.7 t
3-OH	4.66 d (4.9)		—	
5-OH	—		9.21 s	
7-OH	9.14 br s		8.97 s	
3′-OH	8.79 br s		8.71 s	
4′-OH	8.73 br s		8.68 s	
2″-OH	5.17 d (5.2)		4.58 d (7.5)	
3″-OH	5.02 d (4.6)		4.76 d (3)	
4″-OH	4.96 d (5.2)		4.53 d (7.5)	
6″-OH	4.52 t (5.7)		4.37 t (5.7)	

a) Long-range coupling was observed with 2-H in the ^{1}H–^{1}H COSY. *b,f*) Assignments in the literature[3b] were revised, respectively. *c, d*) Long-range coupling was observed with C-5 and with C-8a in the HMBC spectrum, respectively. *e*) Long-range coupling was observed with 2-H in the HMBC spectrum. *g*) Accurate δ values and coupling constants were not obtained because of signal overlapping. In pyridine-d_5, these sugar protons give well-separated signals. *h*) Overlapped with H_2O signal.

From these findings and from a comparison of its optical rotational value with that of known (−)-epiafzelechin-5-*O*-β-D-glucopyranoside ($[\alpha]_D$ −38.3°, MeOH),[6] **1** was concluded to be (−)-epicatechin-5-*O*-β-D-glucopyranoside (**1**).

Compound **6**, colorless needles, mp 150—151 °C, showed $[\alpha]_D^{23}$ −95.3° (MeOH) and its molecular formula was determined to be $C_{16}H_{16}O_{10}$ (M^+, 368) by electron impact mass spectrum (EI-MS) and high-resolution mass spectrum (HR-MS) measurements. Its UV spectrum showed absorption maxima at 226 (log ε, 4.20), 250 sh (4.27), 257 (4.31), 287 (3.81), and 317 nm (3.59), suggestive of a chromone chromophore,[7] and the IR spectrum revealed absorptions due to hydroxyl(s) (3400 cm^{-1}), an ester carbonyl (1740 cm^{-1}), a conjugated carbonyl (1660 cm^{-1}), and an aromatic ring (1620, 1578, and 1500 cm^{-1}).

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, showed the presence of a *cis*-olefin (δ_H 8.01 and 6.23, each d, J=6.1 Hz; δ_C 159.5 and 112.8) and a pair of *meta*-coupled methines (δ_H 6.45 and 6.63, each d, J=2.1 Hz; δ_C 101.9 and 96.9), which could be ascribed to a 5,7-dihydroxychromone group.[8] Moreover, they showed signals due to an anomeric methine (δ_H 5.14, d, J=7.3 Hz; δ_C 102.1) and four oxygenated methines along with those of a methoxyl (δ_H 3.77, s; δ_C 53.8) and an ester carbonyl (δ_C 171.5), suggesting that **6** may be a methyl glucuronate derivative of 5,7-dihydroxychromone.

This assumption was supported by the HMBC spectrum of **6** (Fig. 1). As expected, the carbonyl carbon at δ 184.4 (C-4) showed long-range correlations with 2-H (δ 8.01) and 3-H (δ 6.23), while the ester carbonyl at δ 171.5 was correlated with 5′-H (δ 4.14) of the sugar portion and the methoxyl protons (δ 3.77). On the other hand, the quaternary aromatic carbon at δ 109.3 (C-4a) showed long-range correlations with the protons 3-H (δ 6.23), 6-H, and 8-H (δ 6.45 and 6.63, respectively), whereas the oxygenated quaternary carbon at δ 160.2 (C-8a) was correlated with 8-H and 2-H, confirming the presence of a chromone skeleton. Also, in the HMBC spectrum, the anomeric proton (1′-H) of the glucuronate residue showed a long-range correlation with C-7, which was unequivocally assigned on the basis of its long-range correlation with 6-H and 8-H. It should be noted here that the carbons C-4 (δ 184.4) and C-5 (δ 163.9) show weak but significant correlation peaks with the protons 6-H and 8-H and with 3-H, respectively, which may be attributed to the W-type long-range coupling through four bonds.[9]

From these results, **6** was proved to be 5,7-dihydroxychromone-7-*O*-β-D-glucuronide methyl ester (**6**).

Marioside (**7**) is a very minor component obtained as a colorless powder, $[\alpha]_D^{22}$ +25.6° (MeOH). It revealed quasi-molecular ion peaks at m/z 459 $[M+H]^+$ and 481 $[M+Na]^+$ in the FAB-MS, corresponding to the molecular formula $C_{22}H_{34}O_{10}$. The IR spectrum showed strong absorptions due to hydroxyl group(s) (3430 cm^{-1}), an ester carbonyl (1736 cm^{-1}), and a conjugated ester grouping

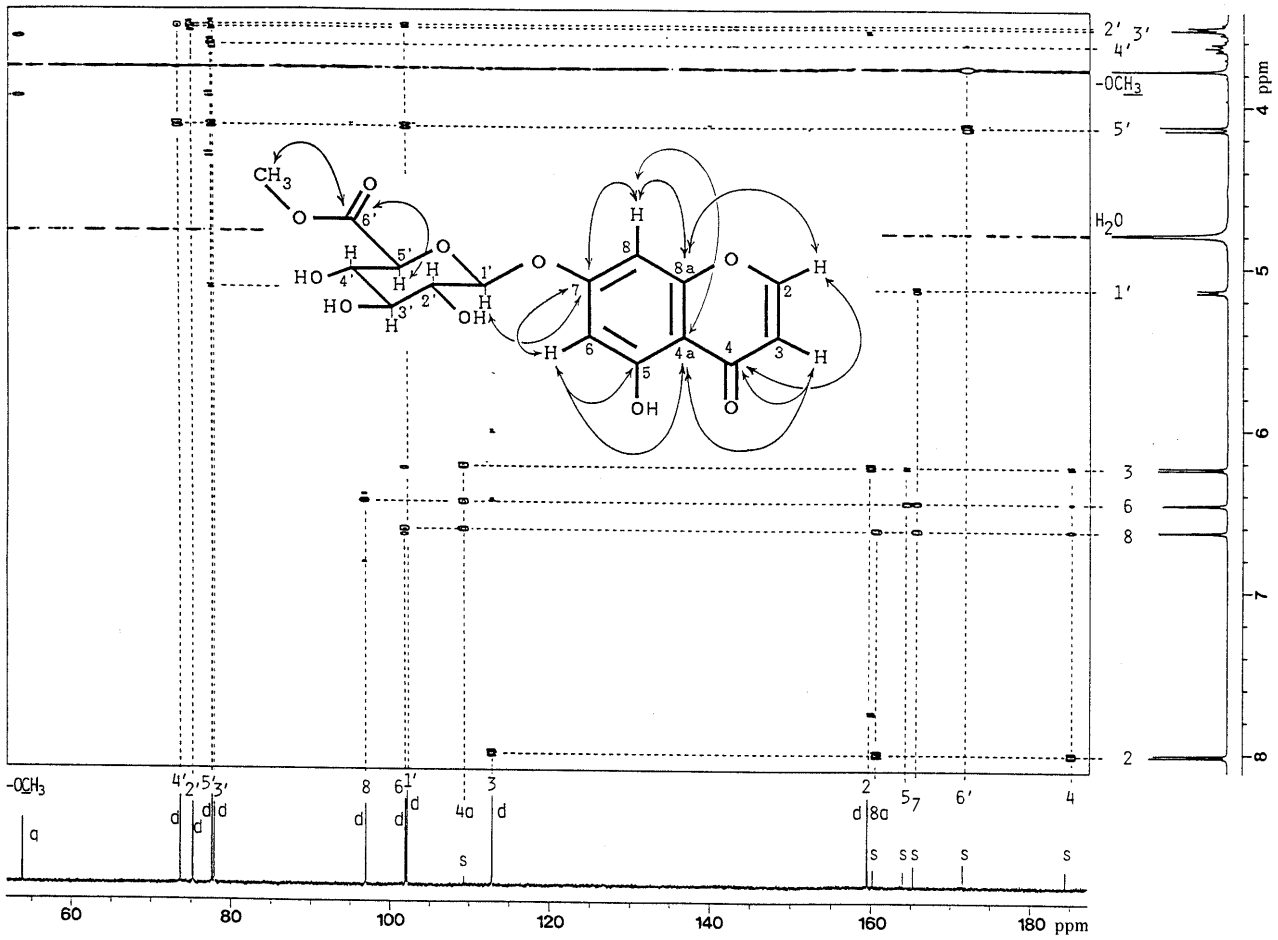

Fig. 1. HMBC Spectrum of 5,7-Dihydroxychromone-7-*O*-*β*-D-Glucuronide Methyl Ester (**6**) in Methanol-d_4
Sample, 13 mg; $^{1r}J_{CH}$ = 8.3 Hz; 12 h run.

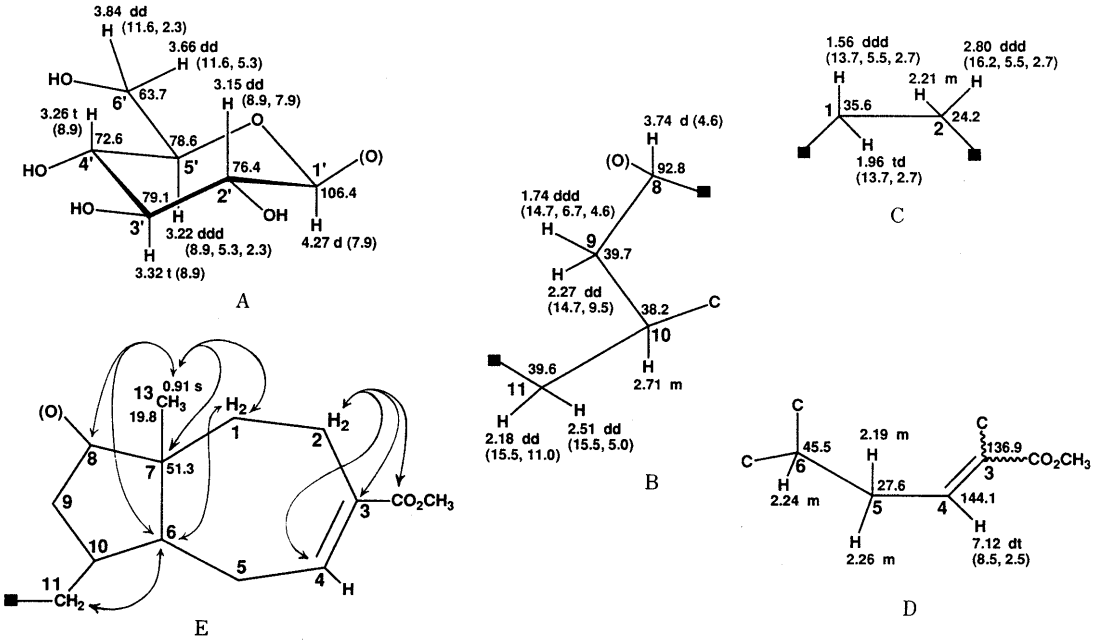

Fig. 2. Partial Structures and NMR Data for Marioside (**7**)
⁓, long-range ^1H–^{13}C correlation in the HMBC spectrum.

(1715 and 1644 cm^{-1}). In the ^1H- and ^{13}C-NMR spectra, **7** showed signals due to a tertiary methyl (δ_H 0.91, s; δ_C 19.8), two methoxyls (δ_H 3.64 and 3.69, each s; δ_C 53.1 and 52.8), and two carbonyls (δ_C 171.7 and 176.6) along with signals of a trisubstituted olefin (δ_H 7.12, dt, J = 8.5, 2.5 Hz; δ_C 144.1 and 136.9). This olefinic group was considered to

be part of an α,β-unsaturated ester group in view of the chemical shift values and the IR data (1715 and 1644 cm^{-1}), as well as the UV absorption at 226 nm (log ε, 3.76).

Further, careful analysis of the ^1H–^1H COSY and the ^1H-detected heteronuclear multiple-quantum coherence (HMQC) spectrum[5b,10] led us to postulate the presence of a glucosyl group (A) and the partial structures B, C, and D depicted in Fig. 2.

Next, in order to deduce the total structure of **7**, we measured the HMBC spectrum. As can be seen in Fig. 3, the tertiary methyl group at δ_H 0.91 shows long-range correlations with the carbons at δ_C 35.6 (C-1 in the partial sructure C), 45.5 (C-6 in the partial structure D), and 92.8 (C-8 in the partial structure B), and also with the quaternary carbon at δ_C 51.3 (C-7). This indicated that the methyl group (δ_H 0.91, δ_C 19.8) should be linked to this quaternary carbon, and the latter to the carbons at δ_C 35.6, 45.5, and 92.8. Also, the methylene protons at δ_H 2.18 and 2.51 (11-H_2 in the partial structure B) show long-range correlations with C-6 (δ_C 45.5, partial structure D), which, in turn, shows long-range correlations with the protons at δ_H 3.74 and 2.27 (8-H and 9_α-H, respectively, in the partial structure B), and δ_H 1.56 (1_β-H in the partial structure C), suggesting that C-10 is connected to C-6. On the other hand, the proton at δ_H 2.80 (2_α-H in the partial structure C) exhibited a correlation with the sp^2 quaternary carbon at δ_C 136.9 (C-3) and also with the olefinic methine carbon at δ_C 144.1 and the ester carbonyl carbon at δ_C 171.7 (C-4 and C-12,

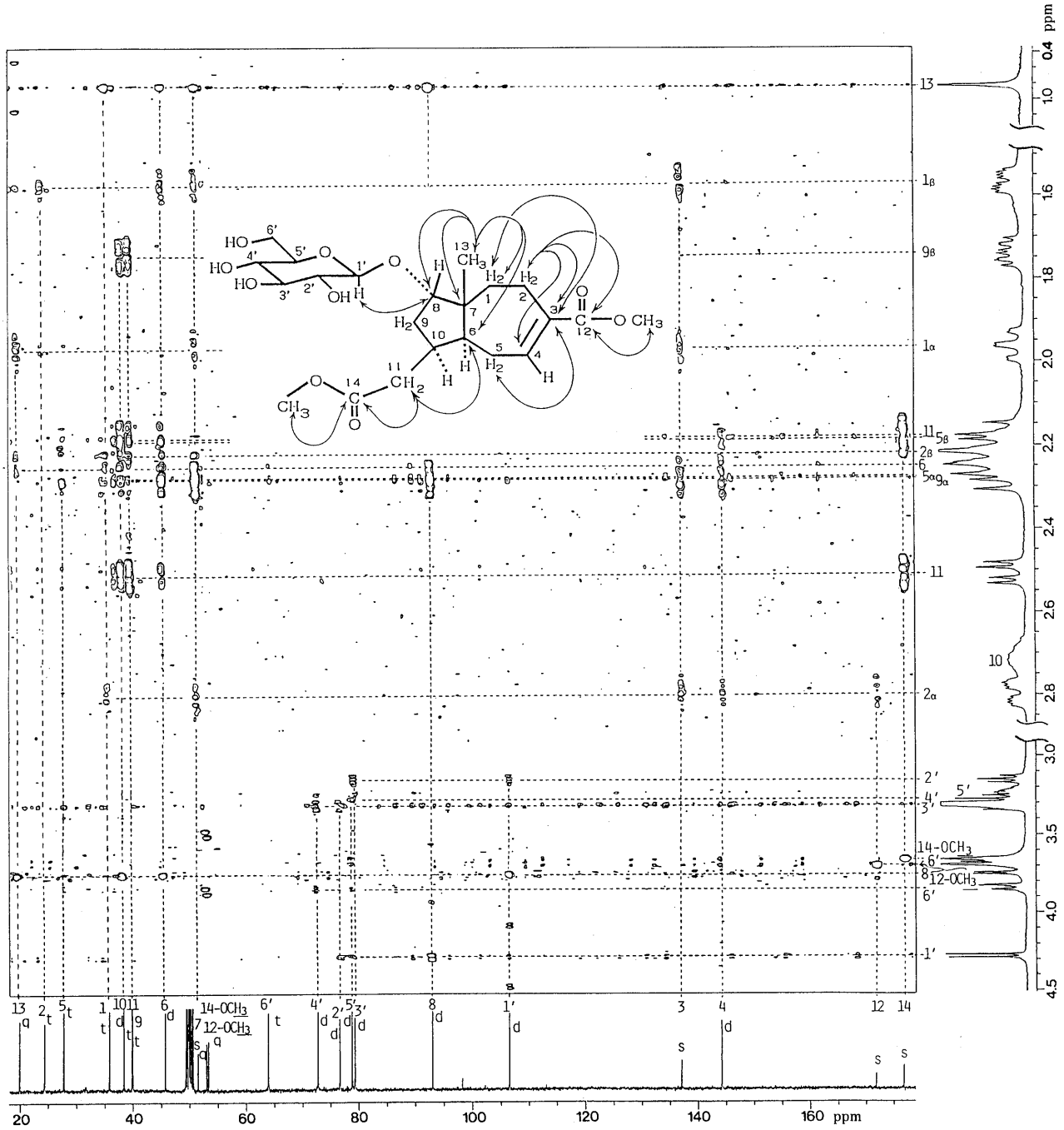

Fig. 3. HMBC Spectrum of Marioside (**7**) in Methanol-d_4

Sample, 4.8 mg; $^{1r}J_{CH}$ = 8.3 Hz; 36 h run.

respectively, in the partial structure C). Thus, the partial structures B, C, and D can be combined to form an expanded structure E.

Another carbonyl carbon at δ_C 176.6 was evidently correlated with 11-H_2 (δ_H 2.18 and 2.51) and the methoxyl protons at δ_H 3.69. Also, long-range correlations between the anomeric carbon (δ_C 104.6) and 8-H and between the anomeric proton (δ_H 4.27) and C-8 were clearly observed. Therefore, the ester group is located at the C-11 position and the β-glucopyranosyl group at the C-8 position.

The relative stereochemistry of **7** was determined based on the results of nuclear Overhauser effect (NOE) experiments. Irradiation of 13-H_3 (δ 0.91) increased the intensities of the 8-H (δ 3.74), 9_β-H (δ 1.74), and 11-H (δ 2.18) signals, suggesting the *cis*-relation of 13-H_3 and 8-H and the *trans*-relation of 13-H_3 and 10-H, whereas irradiation of 10-H (δ 2.71) increased the intensities of 6-H (δ 2.24) and 9_α-H (δ 2.27), suggesting that 10-H and 6-H are in *cis*-relation and the ring junction is *trans*.

From these findings, the structure of marioside was concluded to be a norcarotane sesquiterpene glucoside represented by the formula **7**, except for the absolute stereochemistry.

In conclusion, we have identified three new compounds, (−)-epicatechin-5-*O*-β-D-glucopyranoside (**1**), 5,7-dihydroxychromone-7-*O*-β-D-glucuronide methyl ester (**6**), and marioside (**7**). Among them, **7** is the first example of a norcarotane sesquiterpenoid. Isolation of **6** is of considerable biogenetic interest. Usually, naturally occurring chromones have a methyl or hydroxymethyl substituent at the C-2 position, but a few 2,3-unsubstituted chromone derivatives have been found in nature.[8,11] They are considered to be formed from flavonoids such as eriodictyol, luteolin, and so on.[11b,d] In the present case, **6** may be biosynthesized from eriodictyol-7-*O*-β-D-glucuronide,[12] which co-exists in *D. mariesii*.[2b]

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-4 automatic polarimeter or a JASCO DIP-140 digital polarimeter. UV spectra were taken with a Shimadzu 202 UV spectrophotometer in MeOH solutions and IR spectra on a JASCO IR-2 spectrometer or a Nicolet 5DX FT-IR spectrometer in KBr discs. EI-MS, HR-MS, and FAB-MS were obtained with a JEOL D-300 spectrometer using a direct inlet system and glycerol was used as a matrix in FAB-MS measurements. ^{1}H-, ^{13}C-, and 2D NMR spectra and difference NOE spectra were taken on a JEOL JNM-GX400 spectrometer in methanol-d_4 solutions unless otherwise noted. 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. HPLC separation was carried out on a Shimadzu LC-5A liquid chromatograph using a TSK-GEL ODS-120A (20 × 300 mm) column [solvent, MeOH–H_2O (20:80); flow rate, 9.9 ml/min; detector wavelength, UV_{281} nm].

Isolation of Compounds 1 to 10 The BuOH-soluble fraction (DA-4, 110 g) from the aqueous acetone extract of *Davallia mariesii* MOORE, reported in a previous paper,[2b] was subjected to column chromatography on Sephadex LH-20 (5 × 60 cm) with EtOH–H_2O (1:9 and then 1:3). Fractions were collected in 100 g portions, monitored by TLC, and combined into eight fractions [fr. 1 to fr. 5, EtOH–H_2O (1:9) eluate; fr. 6 to fr.8, EtOH–H_2O (1:3) eluate].

Fraction 3 (2.3 g) was re-chromatographed on a silica gel (50 g) column with $CHCl_3$–MeOH (95:5—50:50) and the eluates were combined into thirteen fractions (fr. 3-1 to fr. 3-13) with monitoring by TLC. Fraction 3-2 [$CHCl_3$–MeOH (95:5) eluate, 20 mg] was further purified by preparative TLC with $CHCl_3$–MeOH (8:2) to give marioside (**7**, 4.8 mg). Fraction 3-4 [$CHCl_3$–MeOH (95:5) eluate, 50 mg] and fr. 3-7 [$CHCl_3$–MeOH (90:10) eluate, 302 mg] were recrystallized from MeOH to give 5,7-dihydroxychromone-7-*O*-β-D-glucuronide methyl ester (**6**, 35.8 mg) and vanillic acid-4-*O*-β-D-glucopyranoside (**5**, 69.8 mg),[4] respectively. Fraction 3-13 [$CHCl_3$–MeOH (50:50) eluate, 173.5 mg] was purified on Sephadex LH-20 column (1.2 × 4 cm) with H_2O to give L-tryptophan (**8**, 103 mg), $[\alpha]_D^{23}$ −22.6° (*c* = 0.5, MeOH).

Fraction 5 (1.5 g) was recrystallized from MeOH to give (−)-epicatechin-5-*O*-β-D-glucopyranoside (**1**, 157 mg). The mother liquor (1.33 g) was chromatographed on a Sephadex LH-20 column (3 × 51 cm) with H_2O and the eluates were combined into eight fractions (fr. 5-1 to fr. 5-8) with monitoring by TLC. Fraction 5-2 (199 mg) was recrystallized from MeOH to give coumaric acid-4-*O*-β-D-glucopyranoside (**3**, 117.5 mg).[2b] Fraction 5-4 (88 mg) was further separated by preparative TLC with AcOEt–EtOH–H_2O (10:2:1) and then by preparative HPLC using a TSK-GEL ODS-120A column (20 × 300 nm) with MeOH–H_2O (2:8) to give davalliosides A (**9**: t_R, 370 min; 24.8 mg) and B (**10**: t_R, 281 min; 12.4 mg).[2a] Fraction 5-6 (186 mg) and fr. 5-7 (50 mg) were recrystallized from MeOH to give caffeic acid-4-*O*-β-D-glucopyranoside (**4**, 111 mg)[2b] and (−)-epicatechin-5-*O*-β-D-glucopyranoside (**1**, 14 mg), respectively.

Fraction 7 (747 mg) was recrystallized from EtOH to give (−)-epicatechin-3-*O*-β-D-allopyranoside (**2**, 458 mg).[3]

(−)-Epicatechin-5-*O*-β-D-glucopyranoside (1) Colorless needles, mp 196—198 °C and 240—241.5 °C, $[\alpha]_D^{25}$ −37.3° (*c* = 0.67, pyridine), $[\alpha]_D^{24}$ −30.6° (*c* = 0.5, MeOH). UV λ_{max} nm (log ε): 212 (4.31), 230 sh (3.97), 281 (3.49). IR ν_{max} cm^{-1}: 3400 (br, OH), 1605, 1500 (aromatic ring), 1070. FAB-MS *m/z*: 453 $[M+H]^+$, 291 [epicatechin+H]$^+$. *Anal.* Calcd for $C_{21}H_{24}O_{11}\cdot H_2O$: C, 53.62; H, 5.57. Found: C, 54.02; H, 5.37. ^{1}H- and ^{13}C-NMR (DMSO-d_6): Table I. ^{1}H-NMR (pyridine-d_5) δ: 5.53 (1H, d, *J* = 7.3 Hz, 1″-H), 4.38 (1H, dd, *J* = 9.5, 7.3 Hz, 4″-H), 4.37 (2H, d, *J* = 3.7 Hz, 6″-H_2), 4.32 (1H, t, *J* = 7.3 Hz, 2″-H), 4.29 (1H, t, *J* = 7.3 Hz, 3″-H), 3.91 (1H, dt, *J* = 9.5, 3.7 Hz, 5″-H).

(−)-Epicatechin-3-*O*-β-D-allopyranoside (2) Colorless needles, mp 165—168 °C and 171—173 °C, $[\alpha]_D^{25}$ −34.5° (*c* = 1.8, MeOH). UV λ_{max} nm (log ε): 216 (4.32), 230 sh (4.22), 281 (3.68). IR ν_{max} cm^{-1}: 3300 (OH), 1605, 1500 (aromatic ring), 1150—1000. FAB-MS *m/z*: 453 $[M+H]^+$, 291 [epicatechin+H]$^+$. *Anal.* Calcd for $C_{21}H_{24}O_{11}\cdot H_2O$: C, 53.62; H, 5.57. Found: C, 53.67; H, 5.65. ^{1}H- and ^{13}C-NMR: Table I.

Vanillic Acid-4-*O*-β-D-glucopyranoside (5) Colorless needles, mp 147—148 °C, $[\alpha]_D^{23}$ −82.6° (*c* = 0.5, MeOH). UV λ_{max} nm (log ε): 216 (4.31), 253 (4.12), 288.5 (3.88). IR ν_{max} cm^{-1}: 3300 (br, OH), 1690 (COOH), 1660, 1510 (aromatic ring), 1270, 1220, 1075. FAB-MS *m/z*: 353 $[M+Na]^+$, 331 $[M+H]^+$. ^{1}H-NMR δ: 7.63 (1H, dd, *J* = 8.2, 1.8 Hz, 6-H), 7.60 (1H, d, *J* = 1.8 Hz, 2-H), 7.20 (1H, d, *J* = 8.2 Hz, 5-H), 5.05 (1H, d, *J* = 7.3 Hz, 1′-H), 3.89 (3H, s, 3-OCH$_3$), 3.88 (1H, dd, *J* = 12.2, 2.1 Hz, 6′-H), 3.70 (1H, dd, *J* = 12.2, 5.5 Hz, 6′-H), 3.54 (1H, dd, *J* = 9.2, 7.3 Hz, 2′-H), 3.49 (1H, dd, *J* = 9.2, 8.2 Hz, 3′-H), 3.47 (1H, ddd, *J* = 9.8, 5.5, 2.1 Hz, 5′-H), 3.41 (1H, dd, *J* = 9.8, 8.2 Hz, 4′-H). ^{13}C-NMR δ: 170.3 (s, COOH), 152.8 (s, C-4), 151.1 (s, C-3), 126.8 (s, C-1), 125.6 (d, C-6), 117.2 (d, C-5), 115.2 (d, C-2), 102.7 (d, C-1′), 79.1 (d, C-5′), 78.6 (d, C-3′),75.5 (d, C-2′), 72.0 (d, C-4′), 63.2 (t, C-6′), 57.5 (q, OCH$_3$). These NMR data were obtained by the use of ^{1}H–^{1}H, ^{1}H–^{13}C, and long-range ^{1}H–^{13}C COSY.

5,7-Dihydroxychromone-7-*O*-β-D-glucuronide Methyl Ester (6) Colorless needles, mp 150—151° C, $[\alpha]_D^{23}$ −95.3° (*c* = 0.5, MeOH). UV λ_{max} nm (log ε): 226 (4.20), 250 sh (4.27), 257 (4.31), 287 (3.81), 317 (3.59). IR ν_{max} cm^{-1}: 3400 (OH), 1740 (ester CO), 1660 (chromone CO), 1620, 1578, 1500 (aromatic ring), 1120—1020 (br). EI-MS *m/z* (%): 368 (M^+, 10), 350 (M^+-H_2O, 3), 191 ([methyl glucuronate−OH]$^+$, 4), 178 ([5,7-dihydroxychromone]$^+$, 100), 173 ([191−H_2O]$^+$, 7), 150 ([178−CO]$^+$, 6), 113 (6). HR-MS: Found 368.0746, Calcd for $C_{16}H_{16}O_{10}$ (M^+) 368.0743; Found 178.0260, Calcd for $C_9H_6O_4$ 178.0255. ^{1}H-NMR δ: 8.01, 6.23 (each 1H, d, *J* = 6.1 Hz, 2-H and 3-H, respectively), 6.63, 6.45 (each 1H, d, *J* = 2.1 Hz, 8-H and 6-H, respectively), 5.14 (1H, d, *J* = 7.3 Hz, 1′-H), 4.14 (1H, d, *J* = 9.7 Hz, 5′-H), 3.77 (3H, s, OCH$_3$), 3.63 (1H, t, *J* = 9.7 Hz, 4′-H), 3.53 (1H, dd, *J* = 9.7, 7.3 Hz, 3′-H), 3.51 (1H, t, *J* = 7.3 Hz, 2′-H). ^{13}C-NMR δ: 184.4 (s, C-4), 171.5 (s, C-6′), 165.2 (s, C-7), 163.9 (s, C-5), 160.2 (s, C-8a), 159.5 (d, C-2), 112.8 (d, C-3), 109.3 (s, C-4a), 102.1 (d, C-1′), 101.9 (d, C-6), 96.9 (d, C-8), 77.8 (d, C-3′), 77.5 (d, C-5′), 75.1 (d, C-2′), 73.6 (d, C-4′), 53.8 (q, OCH$_3$).

Marioside (7) Colorless amorphous solid, $[\alpha]_D^{22}$ +25.6° (c=1.1, MeOH). UV λ_{max} nm (log ε): 226 (3.76). IR ν_{max} cm^{-1}: 3430 (br, OH), 1736 (ester CO), 1715 (conjugated ester CO), 1644 (C=C), 1075, 1033. FAB-MS m/z: 481 $[M+Na]^+$, 459 $[M+H]^+$. ^{1}H-NMR δ: 3.69 (3H, s, 12-OCH$_3$), 3.64 (3H, s, 14-OCH$_3$), 0.91 (s, 13-H$_3$), and Fig. 2. ^{13}C-NMR δ: 176.6 (s, C-14), 171.7 (s, C-12), 53.1 (q, 12-OCH$_3$), 52.8 (q, 14-OCH$_3$), 51.3 (s, C-7), 19.8 (C-13), and Fig. 2.

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References and Notes

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12) Stocker and Pohl[11d] reported that 5,7-dihydroxychromone-7-rutinoside was isolated from *Mentha longifolia*, but it was considered to be a product of postmortem processes because it was only formed after heating fresh plant material. In the present case, the possibility that **6** is an artifact formed from eriodictyol-7-*O*-β-D-glucuronide may be excluded, since **6** was obtained as a methyl ester.