

Constituents of a Fern, *Davallia mariesii* MOORE. I. Isolation and Structures of Davallialactone and a New Flavanone Glucuronide

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A new γ -lactone derivative named davallialactone (**4**) and the 7-*O*- β -D-glucuronide of ($\pm$)-eriodictyol (**5a**) have been isolated from *Davallia mariesii* MOORE along with caffeic acid (**1**), 4-*O*- β -D-glucopyranosylcaffeic acid (**2**) and 4-*O*- β -D-glucopyranosyl-*p*-coumaric acid (**3**). The structures of the new compounds were determined by chemical and spectroscopic methods including two-dimensional nuclear magnetic resonance (2D NMR) techniques, especially ¹H-detected heteronuclear multiple-bond multiple-quantum coherence and long-range C–H *J*-resolved 2D NMR techniques.

Keywords *Davallia mariesii*; Davalliaceae; fern; davallialactone; 7-*O*- β -D-glucuronide of ($\pm$)-eriodictyol; flavanone glucuronide; heteronuclear multiple-bond multiple-quantum coherence; long-range C–H *J*-resolved 2D NMR; 2D NMR

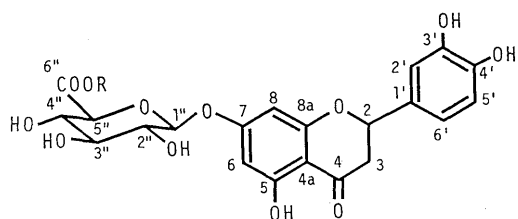
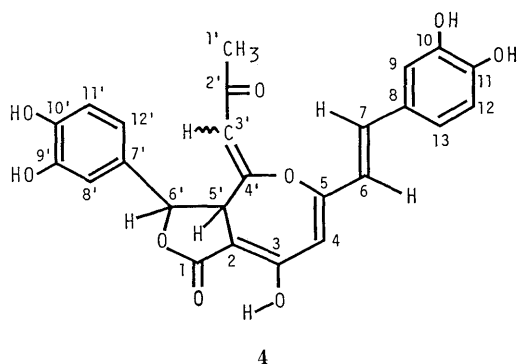
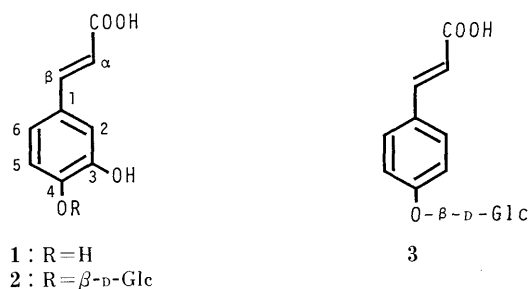
The rhizoma of a fern *Davallia mariesii* MOORE (Davalliaceae) are used in Korea as a folk medicine “Sin Seong Cho (神聖草)” for treatment of common cold, neuralgia, and stomach cancer.¹⁾ In China, the same plant is called “Hai Zhou Gu Sui Bu (海州骨碎補)” and is used as a traditional medicine for treatment of lumbago, rheumatism, odontalgia, tinnitus, and so on.²⁾ Chemical constituents of *Davallia* species have been studied by several groups of authors and the isolation and identification of triterpenes, phenolic compounds, and a cyanogenic compound named vicianin were reported.³⁾ However, until now no work has been done on the constituents of *D. mariesii*. In the course of our chemical studies on biologically active constituents of Korean folk medicines, we investigated the constituents of the rhizoma of *D. mariesii* and isolated a new γ -lactone derivative, named davallialactone (**4**), and a new flavanone glucuronide, the 7-*O*- β -D-glucuronide of ($\pm$)-eriodictyol (**5a**) together with caffeic acid (**1**), 4-*O*- β -D-glucopyranosylcaffeic acid (**2**),⁴⁾ and 4-*O*- β -D-glucopyranosyl-*p*-coumaric acid (**3**).^{4,5)} This paper deals with the isolation and structure elucidation of the new compounds and identification of three known compounds.

The rhizoma of *D. mariesii* were pulverized and extracted successively with methylene chloride, 80% aqueous acetone, and ethanol at room temperature. The aqueous acetone extract was further separated as shown in Chart 2 to give fractions DA-1 to DA-5. Among these, fractions DA-3 and DA-4 showed an inhibitory effect against protein kinase C activity. The fraction DA-3 was subjected to column chromatography over Sephadex LH-20 and eluted with ethanol (EtOH) and then with EtOH–methanol (MeOH) (1:1). The early fractions eluted with EtOH were further separated by Polyamide column chromatography or by a combination of preparative thin-layer chromatography (preparative TLC) and Sephadex LH-20 column chromatography to give compounds **1** to **5a**. Among these, compounds **1** and **3** were identified as caffeic acid (**1**) and 4-*O*- β -D-glucopyranosyl-*p*-coumaric acid (**3**),^{4,5)} respectively.

Compound **2** was obtained as colorless needles, mp 136–137°C, C₁₅H₁₈O₉, [α]_D²⁴ –87.1° (MeOH). This compound was identified as 4-*O*- β -D-glucopyranosylcaffeic acid (**2**)⁴⁾ by detailed analysis of its proton and carbon-13

nuclear magnetic resonance (¹H- and ¹³C-NMR) spectra with the aid of ¹H–¹H shift correlation spectroscopy (COSY) and ¹H-detected heteronuclear multiple quantum coherence (HMQC) and ¹H-detected heteronuclear multiple-bond multiple-quantum coherence (HMBC) spectroscopy.^{6–8)}

Davallialactone (**4**) was obtained as a yellow amorphous powder and showed [α]_D²⁷ +348.4° (MeOH). The elemental



5a: R=H
5b: R=CH₃
Chart 1

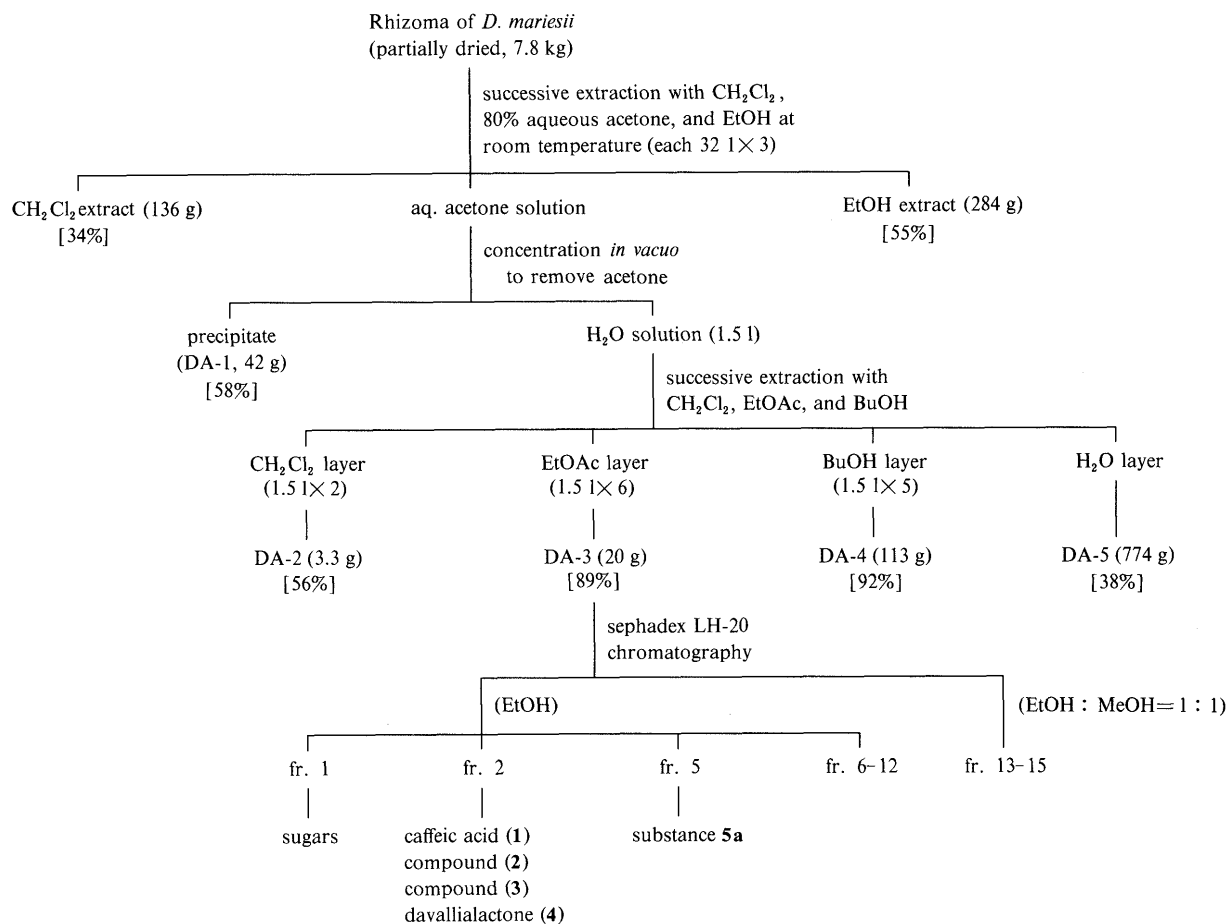
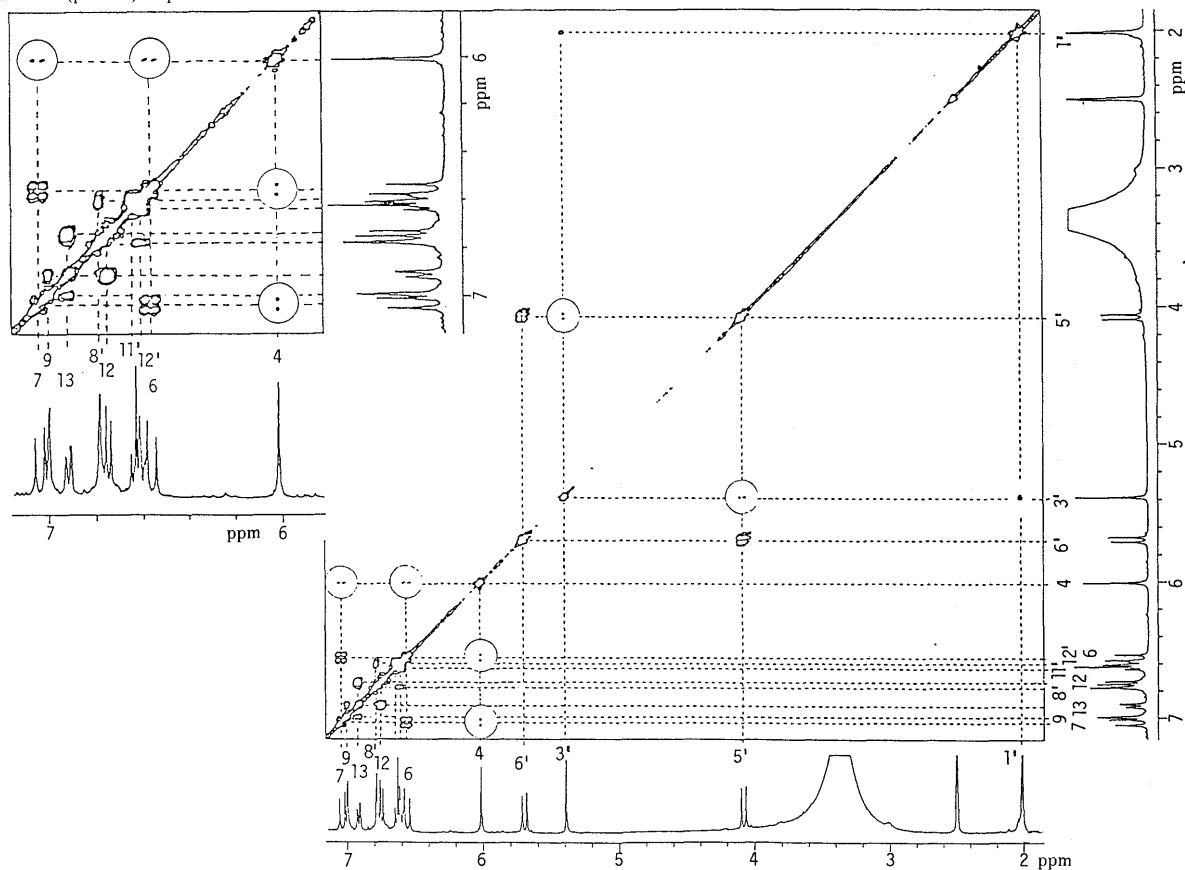
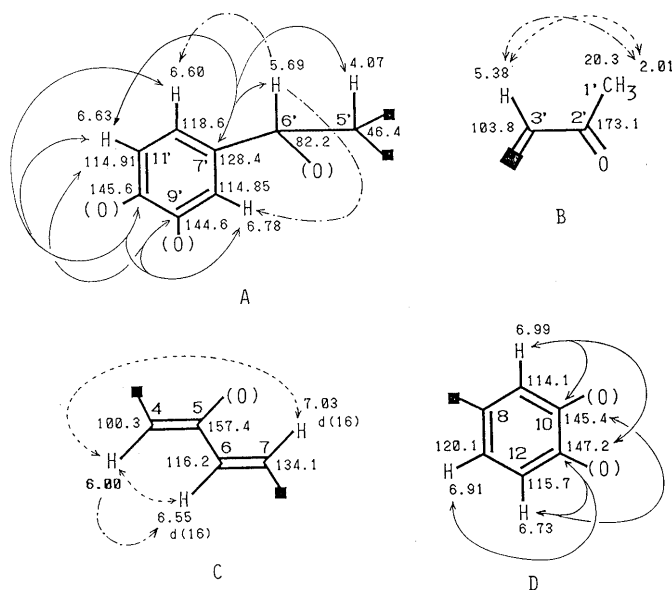
Chart 2. Isolation Scheme of the Constituents of *D. mariesii*[]: Inhibition (percent) of protein kinase C at a concentration of 40 $\mu\text{g}/\text{ml}$.Fig. 1. ^1H - ^1H Shift-Correlation Spectrum of Davallialactone (4) in $\text{DMSO}-d_6$ at 40 $^\circ\text{C}$

TABLE I. 400 MHz ^1H - and 100 MHz ^{13}C -NMR Data for **4** in $\text{DMSO}-d_6$ at 40°C^a

Position	δ_{H} (J in Hz)	δ_{C}	^1H Long-range coupled ($^3J_{\text{CH}}$ $^2J_{\text{CH}}^b$)	
1	—	191.2 s	5', 6'	5'
2	—	97.2 s	4, 6'	5'
3	—	167.6 s	5'	4
4	6.00 ^d brs	100.3 d	6	
5	—	157.4 s	7	4, 6
6	6.55 d (16.0)	116.2 d	4	
7	7.03 d (16.0)	134.1 d	9, 13	6
8	—	126.8 s	6, 12	7
9	6.99 d (2.0)	114.1 d	7, 13	
10	—	145.4 s	12	9
11	—	147.2 s	9, 13	12
12	6.73 d (8.0)	115.7 d		
13	6.91 dd (8.0, 2.0)	120.1 d	7, 9	
1'	2.01 s	20.3 q	3'	
2'	—	173.1 s		1', 3'
3'	5.38 ^d brs	103.8 d	1'	
4'	—	162.9 s		5'
5'	4.07 d (13.4)	46.4 d	3'	6'
6'	5.69 d (13.4)	82.2 d	8', 12'	5'
7'	—	128.4 s	5', 11'	6'
8'	6.78 d (1.5)	114.85 d	6'	12'
9'	—	144.6 s	11'	8'
10'	—	145.6 s	8', 12'	11'
11'	6.63 d (8.0)	114.91 d		12'
12'	6.60 dd (8.0, 1.5)	118.6 d	6', 8'	
OH	11.38 brs (3-OH)	—		
	9.32 brs	—		
	8.94 brs	—		
	8.88 brs	—		
	8.81 brs	—		

a) Signal assignments are based on the results of ^1H - ^1H COSY, ^1H - ^{13}C COSY, and HMBC spectra. b) $^2J_{\text{CH}}$ and $^3J_{\text{CH}}$ indicate the protons coupled with the carbon through two and three bonds, respectively, which were observed in the HMBC spectrum. c) Long-range couplings were observed with 6-H and 7-H in ^1H - ^1H COSY. d) Long-range couplings were observed with 1'-H₃ and 5'-H in ^1H - ^1H COSY.

analysis of **4** was consistent with the molecular formula $\text{C}_{25}\text{H}_{20}\text{O}_9$, which was supported by the appearance of the quasi-molecular ion peaks at m/z 465 $[\text{M} + \text{H}]^+$ and at m/z 463 $[\text{M} - \text{H}]^-$ in the positive and negative ion fast atom bombardment mass spectra (FAB-MS), respectively. It showed a dark color with ferric chloride reagent, suggesting that **4** is a phenolic compound. In the ultraviolet (UV) spectrum, **4** showed absorption bands at 256 ($\log \epsilon$ 4.08) and 364 nm ($\log \epsilon$ 4.07) and in the infrared (IR) spectrum it showed absorptions due to hydroxyl groups (3300 cm^{-1}), conjugated carbonyls (1660 and 1650 cm^{-1}), and aromatic rings (1600 , 1540 , and 1520 cm^{-1}). The ^1H -NMR spectrum of **4** revealed the presence of an acetyl methyl (δ 2.01, s, 1'-H₃), two *vicinal*-oriented methines (δ 4.07 and 5.69, each d, $J=13.4\text{ Hz}$, 5'-H and 6'-H, respectively), two olefinic methines (δ 5.38, brs, 3'-H; δ 6.00, brs, 4'-H), a *trans*-olefin (δ 6.55 and 7.03, each d, $J=16.0\text{ Hz}$; 6-H and 7-H, respectively), two 1,3,4-trisubstituted benzene rings (δ 6.99, d, $J=2.0\text{ Hz}$, 9-H; 6.91, dd, $J=8.0$, 2.0 Hz , 13-H; 6.73, d, $J=8.0\text{ Hz}$, 12-H; δ 6.78, d, $J=1.5\text{ Hz}$, 8'-H; 6.60, dd, $J=8.0$, 1.5 Hz , 12'-H; 6.63, d, $J=8.0\text{ Hz}$, 11'-H) along with a hydrogen-bonded hydroxyl (δ 11.38, brs, 3-OH) and four phenolic hydroxyl groups (δ 9.32, 8.94, 8.88, and 8.81, each brs).⁹⁾ The ^{13}C -NMR spectrum showed two carbonyl carbon signals at δ 191.2 (C-1) and 173.1 (C-2') along with

Fig. 2. Partial Structures and NMR Data for Davallialactone (**4**)

↔ Long-range ^1H - ^{13}C correlation in the HMBC spectrum. - - - NOE observed in difference NOE experiments. ····· Long-range ^1H - ^1H coupling in the ^1H - ^1H COSY spectrum.

signals due to a methyl (δ 20.3, C-1'), two sp^3 methines, (δ 46.4 and 82.2, C-5' and C-6'), ten sp^2 methines, and ten sp^2 quaternary carbons (Table I).

Detailed analysis of these ^1H - and ^{13}C -NMR spectra with the aid of ^1H - ^1H COSY (Fig. 1), ^1H - ^{13}C COSY, and difference nuclear Overhauser effect (NOE) experiments led us to postulate the partial structures A, B, C, and D to be present in **4** (Fig. 2).

Then, we measured the HMBC spectrum of **4** in order to determine the total structure. As can be seen in Fig. 3, the quaternary carbon at δ 144.6 (C-9' in A) shows long-range correlations with the aromatic methine protons at δ 6.63 and 6.78 (11'-H and 8'-H, respectively, in A), while the carbon at δ 145.6 (C-10' in A) shows correlations with the protons at δ 6.60, 6.63, and 6.78 (12'-H, 11'-H, and 8'-H, respectively, in A). Therefore these carbons are assigned to C-9' and C-10', respectively. Similarly, the quaternary carbons at δ 145.4 and 147.2 (C-10 and C-11, respectively, in D) are correlated with the aromatic protons at δ 6.99 (9-H) and 6.73 (12-H) and at δ 6.99 (9-H), 6.91 (13-H), and 6.73 (12-H), respectively, indicating that they can be assigned to C-10 and C-11, respectively. These four quaternary carbons are each considered to carry a hydroxyl group in view of their chemical shift values and the existence of four phenolic hydroxyl groups in **4**.

Further, the methine proton at δ 5.38 (3'-H in B) shows long-range correlations with the methine carbon at δ 46.4 (C-5' in A) and with the methyl and carbonyl carbons (δ 20.3 and 173.1, respectively), while the methine proton at δ 4.07 (5'-H in A) shows correlation with the quaternary carbon at δ 162.9 (C-4') which may be assigned to an oxygenated sp^2 carbon from the chemical shift value. Thus, it is reasonable to connect the structures A and B, leading to an expanded structure E (Fig. 4). This was supported by the ^1H - ^1H COSY (Fig. 1), in which the methine proton at δ 4.07 (5'-H) shows long-range correlation with the olefinic proton at δ 5.38 (3'-H). It should be noted here that the

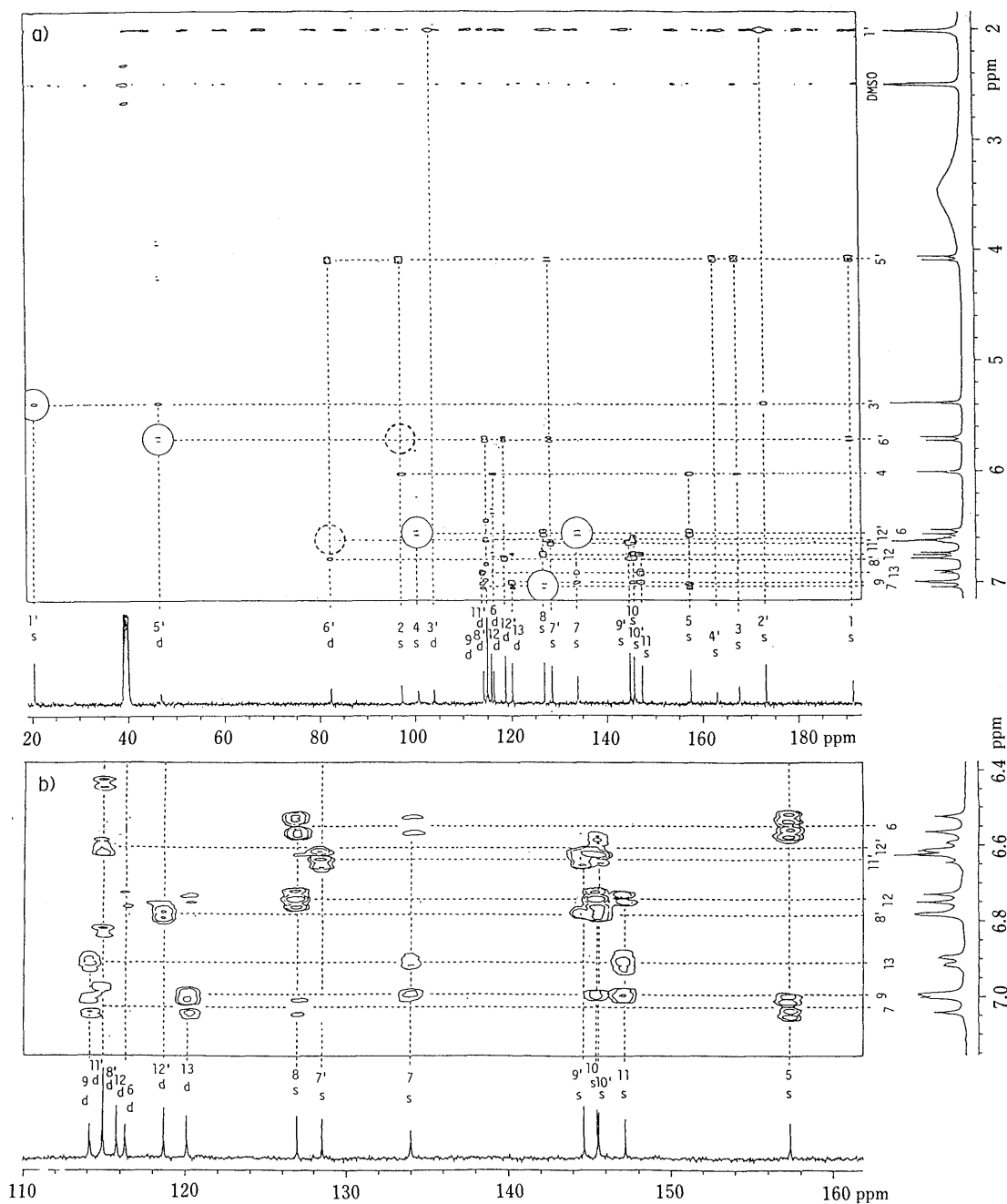


Fig. 3. HMBC Spectrum of Davallialactone (**4**) in DMSO- d_6 at 40 °C (Sample: 22 mg, Long-range J_{CH} =8.3 Hz, 12 h run). a) Whole Region, b) Low Field Region

Open circles indicate significant but weak peaks at this threshold level. Dotted circles indicate the expected signals which were not observed in this experiment, but were observed in another experiment with a value of long-range J_{CH} =2.5 Hz.

acetyl carbonyl carbon appears at fairly high field (δ 173.1), but this may be explained in terms of vinylogous ester structure.¹⁰⁾ On the other hand, the olefinic protons at δ 7.03 and 6.55 (7-H and 6-H, respectively, in C) are correlated with the aromatic carbons at δ 126.8, 120.1, and 114.1 (C-8, C-13, and C-9, respectively, in D) and at δ 126.8 (C-8), respectively, while the aromatic protons at δ 6.99 and 6.91 (9-H and 13-H, respectively, in D) are both correlated with the olefinic carbon at δ 134.1 (C-7 in C). Therefore, the partial structures C and D are connected to give a structure F (Fig. 4).

At this stage, our attention was turned to the remaining three carbon signals (δ 97.2, 167.6, 191.2). In the HMBC spectrum, both the proton signals at δ 4.07 (5'-H in E) and

6.00 (4-H in F) show long-range correlations with a quaternary carbon at δ 97.2 (C-2) and an oxygenated sp^2 carbon at δ 167.6 (C-3), suggesting that the partial structures E and F may be combined across these two carbon atoms. In addition, the methine proton at δ 5.69 (6'-H in E) shows long-range correlation with the quaternary carbon at δ 97.2 (C-2), and further, the methine protons at δ 4.07 and 5.69 (5'-H and 6'-H) both show long-range correlation with the carbonyl carbon at δ 191.2 (C-1). From these findings, it is reasonable to surmise that this carbonyl carbon forms a γ -lactone with an oxygen atom on the methine carbon at δ 82.2 (C-6'), and thus to deduce an expanded partial structure G (Fig. 4).¹¹⁾

Now, in view of the molecular formula ($C_{25}H_{20}O_9$) and

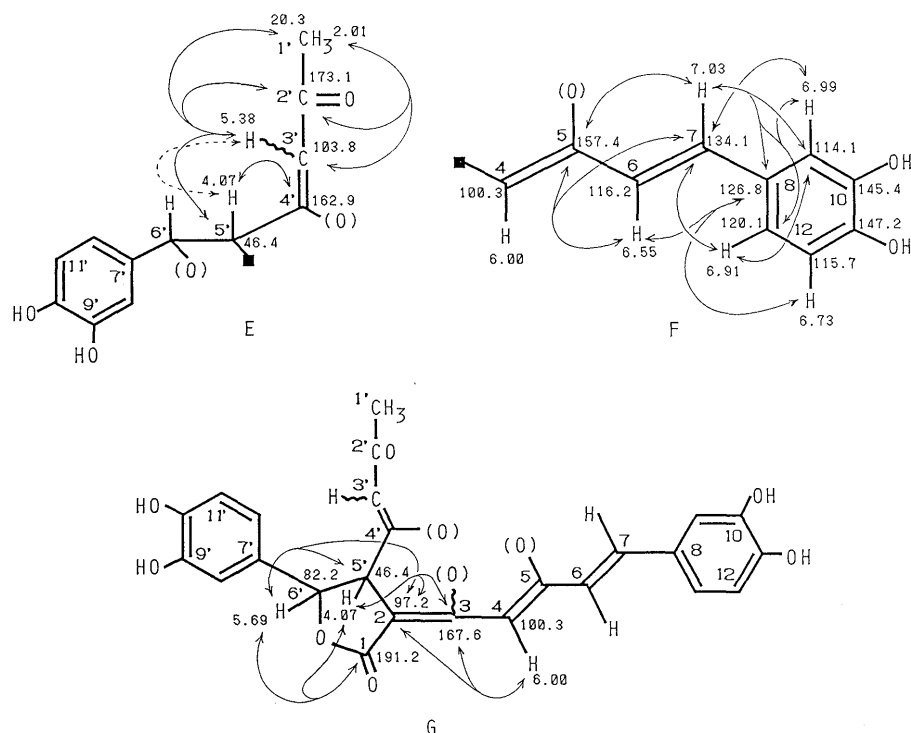


Fig. 4. Partial Structures and NMR Data for Davallialactone (**4**)

→ Long-range ^1H – ^{13}C correlation in the HMBC spectrum. - - - - - Long-range ^1H – ^1H coupling in the ^1H – ^1H COSY spectrum.

the presence of an unspecified hydroxyl group (δ 11.38, br s, in the ^1H -NMR spectrum) in **4**, two of the undefined oxygen atoms in the partial structure G must be forming an ether linkage.

The location of the hydroxyl group was elucidated by the following ^{13}C -NMR experiment under slow OH-OD exchange conditions. As can be seen in Fig. 5, on addition of an H_2O – D_2O mixture (1 : 1), the carbon signal at δ 167.6 (C-3) shows a marked shift and appears as a split broad signal.¹²⁾ Thus the hydroxyl group must be located at this carbon (δ 167.6, C-3) and the ether linkage between two sp^2 carbons at δ 162.9 (C-4') and 157.4 (C-5). This hydroxyl group may be forming a hydrogen bond with the carbonyl carbon at δ 191.2 (C-1).¹³⁾

Based on these findings, we propose the formula **4** for the structure of davallialactone (Chart 1). The stereochemistry at the C-3', C-5', and C-6' positions and the absolute configuration of **4** are currently under investigation.

Substance **5a** was obtained as a pale brown amorphous powder, $[\alpha]_{\text{D}}^{30}$ -45.2° (MeOH), and showed a dark color with ferric chloride reagent and an orange color with anisaldehyde–sulfuric acid reagent. The elemental analysis data of **5a** and the quasi-molecular ion peak in the negative ion FAB-MS ($[\text{M} - \text{H}]^-$; m/z 463) agreed well with the formula $\text{C}_{21}\text{H}_{20}\text{O}_{12}$. It showed UV absorptions at 284 (log ϵ 4.30) and 329 nm (log ϵ 3.65) and IR absorptions at 3400 (OH, very strong), 1643 (conjugated C=O), 1578, and 1520 cm^{-1} (aromatic ring). In the ^1H - and ^{13}C -NMR spectra, it showed signals assignable to an anomeric proton and an anomeric carbon at around δ_{H} 5.2 and δ_{C} 99, respectively, suggesting the presence of a sugar group.

In addition, the IR spectrum of **5a** showed characteristic absorptions at 3200–2400 (br) and 1733 cm^{-1} , which could be ascribed to a carboxyl group. Methylation of **5a** with

diazomethane afforded a methyl ester (**5b**), ν_{max} 1740 cm^{-1} (ester CO), which showed the quasi-molecular ion peak at m/z 477 $[\text{M} - \text{H}]^-$ in the negative ion FAB-MS. In the ^1H – ^{13}C long-range COSY of **5b**, the ester carbonyl carbon (δ 170.1) showed long-range correlations with the methoxy protons (around δ 3.70) and with two protons at around δ 3.70 (4''-H) and 4.23 (5''-H) which may be ascribed to methine protons in the sugar moiety.

Extensive analysis of the ^1H - and ^{13}C -NMR spectra of **5a** and **5b** with the aid of ^1H – ^1H , ^1H – ^{13}C , and long-range ^1H – ^{13}C COSY and ^1H J -resolved two-dimensional nuclear magnetic resonance (2D NMR) led to a suggestion that **5a** may be a flavonoid β -glucuronide. However, it was found that some of the proton and carbon signals appeared as pairs of closely adjacent double lines (Tables II and III). Therefore, **5a** was considered to be a mixture of two isomers.

Treatment of **5a** with 3% aqueous hydrochloric acid (HCl) according to Hori *et al.*¹⁴⁾ gave a crystalline compound, mp 264–266 $^\circ\text{C}$ (dec.), $[\alpha]_{\text{D}}^{32}$ 0° (MeOH), which was identified as ($\pm$)-eriodictyol by comparisons of its spectral data with those published for the (–)-enantiomer.¹⁴⁾ Thus, **5a** was believed to be a diastereomeric mixture of eriodictyol β -glucuronides. However, attempts at the separation of these diastereomers were unsuccessful.

Next, in order to clarify the position of the glucuronide linkage, we measured the long-range C–H J -resolved 2D NMR (LRCJR) spectrum¹⁵⁾ under selective irradiation at 1''-H (Fig. 6). The signals due to C-7 (δ 165.0 and 164.9) were split into doublets ($J = 2.1\text{ Hz}$). Thus, the glucuronide group should be located at the C-7 position of eriodictyol. Since only D-glucuronic acid is known in nature, **5a** was determined to be the 7-O- β -D-glucuronide of ($\pm$)-eriodictyol. This is the first example of a naturally occurring flavanone glucuronide.

Among the compounds described in this paper, **2**, **3**, and **5a** showed a weak inhibitory effect against protein kinase C activity, while **4** showed a weak cytotoxic effect against BALB/3T3 cells transformed by H-ras oncogene (IC₅₀ 21 µg/ml). Details of these biological activities will be reported elsewhere.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured in MeOH solutions on a JASCO DIP-4 automatic polarimeter or a JASCO DIP-140 digital polarimeter at 24–32 °C. UV spectra were taken with a Shimadzu

202 UV spectrophotometer in MeOH and IR spectra were recorded on a JASCO IR-2 spectrometer or a Nicolet 5DX FT-IR spectrometer in KBr discs. Electron impact mass spectrum (EI-MS) (ionization voltage, 70 eV; accelerating voltage, 3 kV) and negative ion FAB-MS were obtained with a JEOL D-300 spectrometer using a direct inlet system and triethanolamine was used as a matrix in negative ion FAB-MS measurements. ¹H- and ¹³C-NMR spectra were taken on a JEOL JNM-GX400 spectrometer with tetramethylsilane as an internal standard and chemical shifts are recorded in δ values. Multiplicities of ¹³C-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). 2D NMR spectra (¹H–¹H COSY, ¹H *J*-resolved 2D NMR, ¹H–¹³C COSY, long-range ¹H–¹³C COSY, HMQC, HMBC, and LRCJR) were measured by the use of JEOL standard pulse sequences and collected data were treated by JEOL standard software. Difference NOE spectra were obtained by the use of a JEOL standard pulse sequence with irradiation for 5 s.

Column chromatography was done with Sephadex LH-20 (Pharmacia) or Polyamide C-100 (40–100 mesh, Wako Pure Chemical Industries, Ltd.). TLC and preparative TLC were carried out on precoated Merck Kieselgel F₂₅₄ plates (0.25 or 0.5 mm) or precoated Merck RP-18 F₂₅₄ reversed-phase plates (0.25 mm) with EtOAc–EtOH–H₂O (20:2:1 or 10:2:1) or MeOH–H₂O (2:3) as the developing solvent, and spots were detected under UV light, or by using FeCl₃ reagent, anisaldehyde–H₂SO₄ reagent, or Ce(SO₄)₂–10% H₂SO₄ (1:99) reagent.

Extraction, Fractionation, and Inhibitory Effects toward Protein Kinase C Partially air-dried rhizoma (7.8 kg) of *Davallia mariesii* MOORE, collected at Pusan, South Korea, in April, 1988, were pulverized and extracted successively with CH₂Cl₂, 80% aqueous acetone, and EtOH (each 32 l × 3, 6 d) at room temperature to afford a CH₂Cl₂ extract (136 g), an aqueous acetone solution, and an EtOH extract (284 g), respectively. The aqueous acetone solution was concentrated under reduced pressure to remove acetone and the precipitate formed (DA-1, 42 g) was collected by filtration. The filtrate (1.5 l) was extracted successively with CH₂Cl₂ and EtOAc, and then with BuOH (each 1.5 l). Each extract and the remaining water layer were evaporated to dryness *in vacuo* to give fractions DA-2 (3.3 g), DA-3 (20 g), DA-4 (112 g), and DA-5 (774 g), respectively.

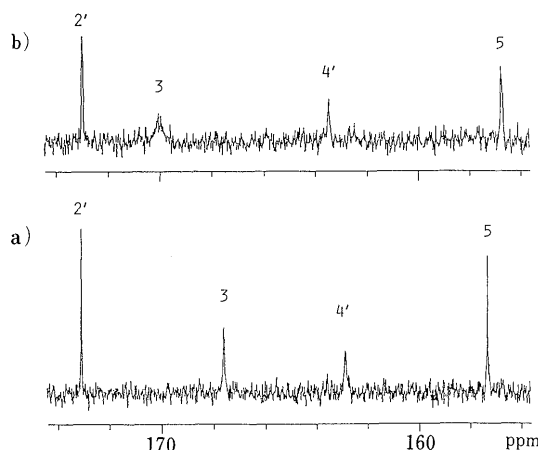


Fig. 5. ¹³C-NMR Spectra of Davallialactone (**4**) in DMSO-*d*₆ at 40 °C. a) Normal Spectrum, b) Spectrum after Addition of a 1:1 mixture of H₂O and D₂O (3 drops)

TABLE II. 400 MHz ¹H-NMR Data (δ ppm, *J* Values in Parenthesis in Hz) for **5a** and **5b**^{a)}

Proton	5a ^{b)}		5a ^{c)}		5b ^{d)}	
2-H	5.46 br dd (12.5, 2.5)	5.43 br dd (12.5, 2.5)	5.28 dd (12.8, 3.1)	5.27 dd (12.8, 3.1)	5.44 dd (12.8, 3.1)	5.33 dd (12.8, 3.1)
3-H _a	3.27 dd (16.9, 12.5)		3.10 dd (17.0, 12.8)	3.09 dd (17.0, 12.8)	3.21 dd (17.1, 12.8)	3.20 dd (17.1, 12.8)
3-H _c	2.75 dd (16.9, 2.5)	2.74 dd (16.9, 2.5)	2.72 dd (17.0, 3.1)	2.71 dd (17.0, 3.1)	2.77 dd (17.1, 3.1)	2.76 dd (17.1, 3.1)
6-H	6.17 d (2.3)	6.16 d (2.3)		6.16 d (2.1)	6.14 d (1.8)	6.13 d (1.8)
8-H	6.21 d (2.3)	6.20 d (2.3)		6.17 d (2.1)		6.17 d (1.8)
2'-H	6.92 s	6.91 s		6.92 br s		7.04 br s
5'-H				6.78 br s		6.87 br s
6'-H				6.78 br s		6.87 br s
1''-H	5.19 d (7.5)	5.17 d (7.5)	5.07 d (7.5)	5.06 d (7.5)	5.27 d (7.6)	5.25 d (7.6)
2''-H		3.28 dd (8.5, 7.5)		3.51 dd (8.0, 7.5)		3.54 dd (8.5, 7.6)
3''-H		3.34 dd (9.4, 8.5)		3.53 t (8.0)		3.61 dd (9.2, 8.5)
4''-H		3.41 t (9.4)		3.62 dd (9.5, 8.0)	3.69 t (9.2)	3.70 t (9.2)
5''-H	4.02 d (9.4)	4.01 d (9.4)	4.05 d (9.5)	4.03 d (9.5)	4.24 d (9.2)	4.23 d (9.2)
CO ₂ CH ₃					3.71 s	3.70 s
3'-OH		9.09 br s				8.18 br s
4'-OH		9.09 br s				8.23 br s
5'-OH		12.06 s			12.07 s	12.06 s
2''-OH						4.93 br s
3''-OH						4.65 br s
4''-OH						4.68 br s

a) Signal assignments are based on the results of ¹H–¹H COSY, ¹H homonuclear *J*-resolved 2D NMR, ¹H–¹³C COSY, and long-range ¹H–¹³C COSY experiments. b, c, and d) Values in DMSO-*d*₆, methanol-*d*₄ and acetone-*d*₆, respectively.

TABLE III. 100 MHz ^{13}C NMR Data for **5a** and **5b**^{d)}

Compd.	5a ^{b)}				5a ^{c)}				5b ^{d)}			
^{13}C	δ	^1H Long-range coupled		δ	^1H Long-range coupled		δ	^1H Long-range coupled		δ	^1H Long-range coupled	
		$(^3J_{\text{CH}} \quad ^2J_{\text{CH}})^{\text{e)}$			$(^3J_{\text{CH}} \quad ^2J_{\text{CH}})^{\text{e)}$			$(^3J_{\text{CH}} \quad ^2J_{\text{CH}})^{\text{e)}$			$(^3J_{\text{CH}} \quad ^2J_{\text{CH}})^{\text{e)}$	
2	79.00 d	78.95 d		81.4 d	81.3 d	2'	3 _{ax.}	80.7 d	80.6 d			
3	42.4 t			44.9 t	44.8 t			44.02 t	43.99 t			
4	197.4 s	197.3 s		199.3 s		2	3 _{ax.} , 3 _{eq.}	198.5 s	198.4 s			
4a	103.6 s		6,8,5-OH	105.1 s		6,8,5-OH		105.1 s		6,8,5-OH	3 _{ax.} , 3 _{eq.}	
5	163.1 s		6, 5-OH	165.6 s	165.5 s		6	165.10 s	165.07 s	6	5-OH	
6	96.5 d		5-OH	98.61 d	98.60 d		6	97.9 d		8, 5-OH		
7	165.0 s	164.9 s	1'' ^{f)}	167.23 s	167.18 s		6, 8	166.6 s		1''	6, 8	
8	95.5 d	95.4 d	6	97.71 d	97.66 d	6		96.7 d		6		
8a	163.0 s	162.9 s	2 ^{f)}	165.2 s		8		164.6 s	164.5 s		8	
1'	129.4 s		3 _{ax.} , 3 _{eq.} , ^{f)} 5'	132.18 s	132.15 s	5'	2', 6'	131.7 s		3 _{ax.} , 5'	6'	
2'	114.6 d		2	115.5 d		2', 6'		115.2 d		2, 3'-OH		
3'	145.4 s		5'	147.1 s		5'	2'	146.5 s		5'	2'	
4'	146.0 s		2', 6'	157.6 s		2', 6'	5'	147.0 s		2', 6'	5'	
5'	115.6 d			117.0 d				116.5 d		4'-OH		
6'	118.3 d		2, 2'	120.1 d		2, 2'		119.8 d		2, 2'		
1''	99.1 d	99.0 d		101.64 d	101.61 d	5''		101.1 d	100.9 d	4'', 5''		
2''	72.9 d			75.0 d				74.5 d				
3''	75.7 d			77.8 d				77.3 d				
4''	71.4 d			73.6 d				73.0 d				
5''	75.5 d			77.2 d				76.8 d				
6''	170.2 s		4''	172.80 s	172.79 s	5''		170.1 s		4'', OCH ₃	5''	
-COCH ₃	—			—				52.9 q				

a) Signal assignments are based on the results of ^1H - ^{13}C COSY and long-range ^1H - ^{13}C COSY. b, c, and d) Values in DMSO- d_6 , methanol- d_4 , and acetone- d_6 , respectively. e) $^2J_{\text{CH}}$ and $^3J_{\text{CH}}$ indicate the protons coupled with the carbon through two and three bonds, respectively, which were observed in the long-range ^1H - ^{13}C COSY and long-range C-H J -resolved 2D NMR spectra. f) Long-range coupling was confirmed by LRCJR experiments under selective irradiation at the respective proton signals.

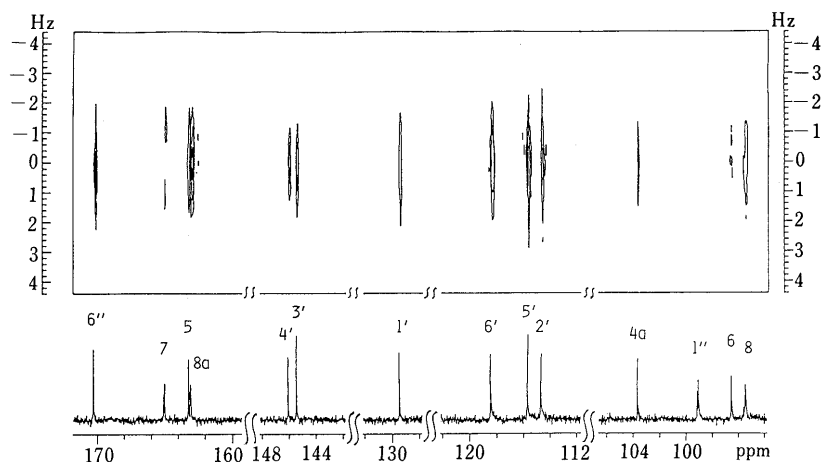


Fig. 6. Long-range C-H J -Resolved 2D NMR Spectrum of the 7- O - β -D-Glucuronide of (+)-Eriodictyol (**5a**) in DMSO- d_6 under Selective Irradiation of 1''-H at around δ 5.18

Inhibitory activity of each extract or fraction toward protein kinase C was measured with histone H1 as a substrate according to the reported method,¹⁶⁾ and the results are shown in Chart 2.

Isolation of 1 to 5a from Fraction DA-3 Fraction DA-3 (20 g) was subjected to column chromatography on Sephadex LH-20 (bed, 5×54 cm) and eluted successively with EtOH (12.6 l) and EtOH-MeOH (1:1, 15 l). Fractions were collected in 5 ml portions and they were monitored by TLC and finally divided into fifteen fractions [fr. 1 to fr. 12: EtOH eluate, fr. 13 to fr. 15: EtOH-MeOH (1:1) eluate].

A portion (446 mg) of fr. 2 (total 1.48 g) was rechromatographed on a Polyamide column (0.4 g; bed, 1.2×17 cm). Elution with H_2O gave a crystalline substance (70 mg), which was recrystallized from MeOH to give caffeic acid (**1**) (14 mg) as pale brown needles. After successive elution of the column with H_2O -MeOH (50:50), further elution with H_2O -MeOH (25:75) gave davallialactone (**4**) (22 mg), which was precipitated from MeOH.

Another portion (259 mg) of fr. 2 was separated by preparative TLC with EtOAc-EtOH- H_2O (20:2:1). The least polar fraction gave caffeic

acid (**1**) (4 mg) and the next least polar fraction davallialactone (**4**) (46 mg). On the other hand, the most polar fraction (22.4 mg) was further purified by reversed-phase preparative TLC with MeOH- H_2O (2:3) to give a crystalline substance, which was subjected again to chromatography on a Sephadex LH-20 column and eluted with H_2O to give compound **3** (7 mg) and compound **2** (7 mg).

Fraction 5 gave substance **5a** (1.02 g) as a pale brown amorphous powder.

Caffeic Acid (1) Pale brown needles (from MeOH), mp 196–197°C. ^1H -NMR (methanol- d_4) δ : 6.23 (1H, d, $J = 15.9$ Hz, α -H), 6.78 (1H, d, $J = 8.2$ Hz, 5-H), 6.93 (1H, dd, $J = 8.2, 2.0$ Hz, 6-H), 7.04 (1H, d, $J = 2.0$ Hz, 2-H), 7.53 (1H, d, $J = 15.9$ Hz, β -H). ^{13}C -NMR (methanol- d_4) δ : 115.9 (d, C-5), 116.4 (d, C-2), 117.3 (d, C- α), 171.9 (s, COOH), 123.6 (d, C-6), 128.6 (s, C-1), 147.5 (s, C-3), 147.8 (d, C- β), 150.2 (s, C-4). The ^1H - and ^{13}C -NMR data were identical with those of an authentic sample.

4- O - β -D-Glucopyranosylcaffeic Acid (2) Colorless needles (from MeOH), mp 136–137°C, $[\alpha]_{\text{D}}^{24} -87.1^\circ$ ($c = 0.7$, MeOH). UV λ_{max} nm (log ϵ): 217 (4.13), 236 (4.04), 287 (4.17), 315 (4.08). IR ν_{max} cm^{-1} : 3250 (OH), 3200–2400 (br, COOH), 1660 (COOH), 1605, 1500 (aromatic ring),

1280, 1070, 860, 800. Negative ion FAB-MS m/z : 341 $[M-H]^-$. 1H -NMR (methanol- d_4) δ : 7.56 (1H, d, $J=15.9$ Hz, β -H), 7.16 (1H, d, $J=8.3$ Hz, 5-H), 7.10 (1H, d, $J=2.1$ Hz, 2-H), 7.03 (1H, dd, $J=8.3$, 2.1 Hz, 6-H), 6.31 (1H, d, $J=15.9$ Hz, α -H), 4.85 (1H, d, $J=7.3$ Hz, 1'-H), 3.91 (1H, dd, $J=11.2$, 1.8 Hz, 6'-H), 3.72 (1H, dd, $J=11.2$, 5.1 Hz, 6'-H), 3.53 (1H, t, $J=7.3$ Hz, 3'-H), 3.51 (1H, t, $J=7.3$ Hz, 2'-H), 3.42 (1H, dd, $J=9.0$, 7.3 Hz, 4'-H), 3.45 (1H, m, 5'-H). ^{13}C -NMR (methanol- d_4) δ : 171.4 (s, COOH), 149.5 (s, C-4; long-range-correlated with 1'-H, 2-H, and 6-H in the HMBC spectrum), 149.3 (s, C-3; long-range-correlated with 5-H in the HMBC spectrum), 146.9 (d, C- β), 131.9 (s, C-1), 122.9 (d, C-6), 118.9 (d, C- α), 118.5 (d, C-5), 116.7 (d, C-2), 104.3 (d, C-1'), 79.1 (d, C-5'), 78.3 (d, C-2'), 75.5 (d, C-3'), 72.0 (d, C-4'), 63.2 (t, C-6').

4-O- β -D-Glucopyranosyl-*p*-coumaric Acid (3) Colorless needles (from MeOH), mp 191–192.5°C, $[\alpha]_D^{25} -72.6^\circ$ ($c=0.7$, MeOH). UV λ_{max} nm (log ϵ): 223 (3.96), 288.5 (4.15), 297 sh (4.14). IR ν_{max} cm^{-1} : 3350 (OH), 3200–2400 (br, COOH), 1675 (COOH), 1600, 1500 (aromatic ring), 1245, 1080, 825. Negative ion FAB-MS m/z : 325 $[M-H]^-$. 1H -NMR (methanol- d_4) δ : 7.62 (1H, d, $J=16.0$ Hz, β -H), 7.55 (2H, d, $J=8.5$ Hz, 2,6-H), 7.12 (2H, d, $J=8.5$ Hz, 3,5-H), 6.36 (1H, d, $J=16.0$ Hz, α -H), 4.96 (1H, d, $J=7.5$ Hz, 1'-H), 3.90 (1H, dd, $J=12.1$, 2.1 Hz, 6'-H), 3.70 (1H, dd, $J=7.5$, 7.0 Hz, 2'-H), 3.46 (1H, ddd, $J=9.0$, 5.5, 2.1 Hz, 5'-H), 3.40 (1H, dd, $J=9.0$, 7.0 Hz, 4'-H). ^{13}C -NMR (methanol- d_4) δ : 171.4 (s, COOH), 161.6 (s, C-4), 146.7 (d, C- β), 131.5 (2 $\times$ C, d, C-2,6), 130.8 (s, C-1), 118.8 (2 $\times$ C, d, C-3,5), 118.2 (d, C- α), 102.6 (d, C-1'), 79.0 (d, C-5'), 78.7 (d, C-2'), 75.6 (d, C-3'), 72.1 (d, C-4'), 63.3 (t, C-6').

Davallialactone (4) Yellow amorphous powder (from MeOH), $[\alpha]_D^{27} +348.4^\circ$ ($c=0.5$, MeOH). UV λ_{max} (log ϵ): 256 (4.08), 292 sh, 335 sh, 364 (4.07). IR ν_{max} cm^{-1} : 3300 (OH), 1660, 1650 (CO), 1640 (C=C), 1600, 1540 (aromatic ring), 1450, 1280, 1160. Positive ion FAB-MS m/z : 465 $[M+H]^+$. Negative ion FAB-MS m/z : 463 $[M-H]^-$. Anal. Calcd for $C_{25}H_{20}O_9 \cdot 3/2H_2O$: C, 61.10; H, 4.71. Found: C, 61.08; H, 4.40. 1H - and ^{13}C -NMR (DMSO- d_6): see Table I.

Substance 5a (7-O- β -D-Glucuronide of ($\pm$)-Eriodictyol) Pale brown powder, $[\alpha]_D^{30} -45.2^\circ$ ($c=1.0$, MeOH). UV λ_{max} nm (log ϵ): 284 (4.30), 329 (3.65). IR ν_{max} cm^{-1} : 3400 (OH), 3200–2400 br (COOH), 1730 (CO), 1640 (COOH), 1615, 1580, 1520 (aromatic ring), 1450, 1200, 1180. EI-MS m/z : 288 $[genin]^+$, 176. Negative ion FAB-MS m/z : 463 $[M-H]^-$. Anal. Calcd for $C_{21}H_{20}O_{12}$: C, 54.31; H, 4.34. Found: C, 54.13; H, 4.25. 1H - and ^{13}C -NMR: see Tables II and III.

Methylation of 5a 5a (25 mg) was methylated with excess CH_2N_2 in ether and the crude product (26 mg) was purified by preparative TLC [MeOH- CH_2Cl_2 (2:8)] to afford a methyl ester (5b) (19.4 mg), amorphous powder, $[\alpha]_D^{32} -89.1^\circ$ ($c=1.2$, MeOH). UV λ_{max} nm (log ϵ): 283 (4.20), 330 (3.64). IR ν_{max} cm^{-1} : 3350 (OH), 1740, 1630 (CO), 1580, 1510 (aromatic ring), 1445, 1380, 1280, 1200, 1170. Negative ion FAB-MS m/z : 477 $[M-H]^-$. 1H - and ^{13}C -NMR (acetone- d_6): see Tables II and III.

Acid Hydrolysis of 5a 5a (100 mg) was refluxed with 3% aqueous HCl solution (8 ml) for 1 h.¹⁴ The reaction mixture was extracted with EtOAc, and the extract was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give a syrup. The residue was separated by preparative TLC with EtOAc-EtOH- H_2O (10:2:1) to give ($\pm$)-eriodictyol (14 mg), yellow needles (from MeOH), mp 264–266°C (dec.), $[\alpha]_D^{32} 0^\circ$ ($c=1.0$, MeOH). UV λ_{max} nm (log ϵ): 215 (4.35), 224 sh (4.31), 288 (4.28), 329 sh (3.78). IR ν_{max} cm^{-1} : 3350 (OH), 1635 (CO), 1605, 1480 (aromatic ring), 1450, 1210, 1160. EI-MS m/z : 288 (M^+ , base peak), 153, 136, 123. 1H -NMR (methanol- d_4) δ : 2.69 (1H, dd, $J=17.1$, 3.1 Hz, 3- H_c), 3.06 (1H, dd, $J=17.1$, 12.8 Hz, 3- H_a), 5.27 (1H, dd, $J=12.8$, 3.1 Hz, 2-H), 5.88 (1H, d, $J=2.1$ Hz, 6-H), 5.90 (1H, d, $J=2.1$ Hz, 8-H), 6.78 (1H, AB type, 5'-H), 6.79 (1H, AB type, 6'-H), 6.91 (1H, brs, 2'-H). ^{13}C -NMR (methanol- d_4) δ : 81.3 (d, C-2), 44.9 (t, C-3), 198.5 (s, C-4), 104.1 (s, C-4a), 166.2 (s, C-5), 97.8 (d, C-6), 169.2 (s, C-7), 97.0 (d, C-8), 165.6 (s, C-8a), 132.5 (s, C-1'), 115.5 (d, C-2'), 147.3 (s, C-3'; long-range-correlated with 2'-H and 5'-H in the long-range 1H - ^{13}C COSY), 147.6 (C-4'; long-range-correlated with 2'-H, 5'-H, and 6'-H in the long-range 1H - ^{13}C COSY), 117.1 (d, C-5'), 120.0 (d, C-6').

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