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# DAVALLIOSIDE A AND B, NOVEL FLAVAN-3-OL DERIVATIVES WITH A γ-LACTAM, FROM THE RHIZOMES OF DAVALLIA MARIESII MOORE[1]

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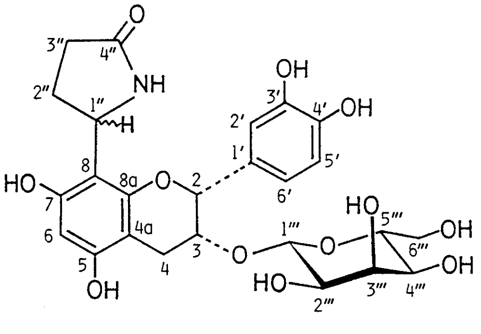
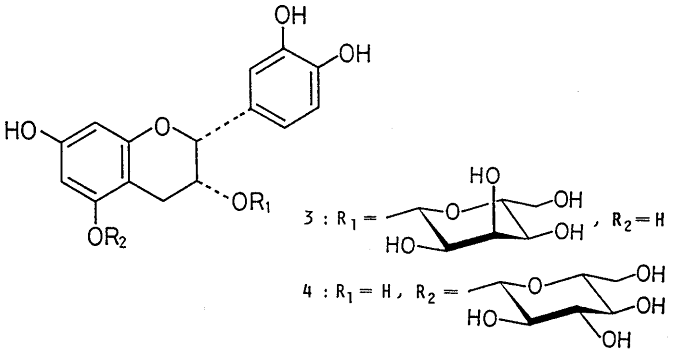
Davallioside A and B, new (-)-epicatechin glycosides carrying a γ-lactam substituent, have been isolated from the rhizomes of Davallia mariesii Moore (Davalliaceae). Their structures were determined by 2D NMR spectroscopy including HMQC and HMBC techniques.

KEYWORDS Davallioside A; Davallioside B; Davalliaceae; Davallia mariesii; 8-(2-pyrrolidinone-5-yl)-(-)-epicatechin-3-O-β-D-allopyranoside; 2D NMR; HMQC; HMBC

In the preceding paper,[2] we reported the isolation and structure of a new compound, davallialactone, from the rhizomes of Davallia mariesii Moore (Davalliaceae), which is used in Korea as a folk medicine "Sin Seong Cho" to treat common colds, neuralgia, and stomach cancer.[3] In a continued study, we have recently isolated two minor flavan-3-ol derivatives, named davallioside A (**1**) and B (**2**). Here we describe the structures of **1** and **2** found by 2D NMR techniques.



1 and 2



The BuOH-soluble fraction (110 g) of the aqueous acetone extract of the dried rhizomes (7.8 kg)[2] was separated by repeated column chromatography on Sephadex LH-20 to give (-)-epicatechin-3-O-β-D-allopyranoside[4] (**3**, 458 mg), a new compound identified as (-)-epicatechin-5-O-β-D-glucopyranoside (**4**, 157 mg), and a mixture fraction (88 mg). The last one was further separated by preparative TLC [Merck Kieselgel 60 $F_{254}$ plates; EtOAc-EtOH-$H_2O$ (10:2:1)] followed by HPLC separation [column, TSK-GEL ODS-120A, 21.5 mm x 35 cm; solvent, MeOH-$H_2O$ (2:8); flow rate, 9.9 ml/min] to give davallioside A (**1**, $t_R$ 370 min, 24.8 mg) and B (**2**, $t_R$ 281 min, 12.4 mg).

Davallioside A (**1**), amorphous solid, showed $[\alpha]_D^{23}$+10.2° (MeOH) and its molecular formula was determined to be $C_{26}H_{29}NO_{12}$ by elemental analysis and FAB-MS measurement (m/z: 536 $[M+H]^+$). It showed UV absorptions at 230sh and 281 nm (log ε : 4.37 and 3.79) and IR (KBr) absorptions at 3350-3200 (br, OH), 1650 (amide CO), 1605, and 1510 $cm^{-1}$ (aromatic ring). The $^1H$- and $^{13}C$-NMR spectra of **1** were similar to those of **3**, but they were characterized by the disappearance of a $^1H$-signal due to one of the ring-A protons and the appearance of new signals assignable to a $CHCH_2CH_2$ residue ($\delta_H$5.39, 2.28, 2.47, 2.33, and 2.37; $\delta_C$51.1, 33.0, and 27.7) and a carbonyl group ($\delta_C$182.3).

Detailed analysis of the $^1H$-$^1H$ COSY and HMQC[5] spectra of **1** (see Table I), coupled with the carbonyl absorption in the IR spectrum, led us to consider that **1** may be an epicatechin-3-O-β-D-allopyranoside derivative carrying a 2-pyrrolidinone-5-yl group at the 6- or 8- position.

The position of 2-pyrrolidinone-5-yl group was determined by the analysis of the HMBC[5] spectrum (Fig.

Table I. 400 MHz $^1$H- and 100 MHz $^{13}$C-NMR Data for **1**, **2**, and **3** in Methanol-$d_4$[a]

| Compd. | **1** | | | | **2** | | | | **3** | |
|---|---|---|---|---|---|---|---|---|---|---|
| Position | $\delta_H$ (J Hz) | $\delta_C$ | $^1$H L.r. coupled[b] ($^3J_{CH}$ | $^2J_{CH}$) | $\delta_H$ (J Hz) | $\delta_C$ | $^1$H L.r. coupled[b] ($^3J_{CH}$ | $^2J_{CH}$) | $\delta_H$ (J Hz) | $\delta_C$ |
| 2 | 5.13 d (2.1) | 80.1 d | 2', 4, 6' | | 5.08 d (2.1) | 80.2 d | 2', 4, 6' | | 5.08 d (2.2) | 79.9 d |
| 3 | 4.42[c] ddd (5.5, 4.6, 2.1) | 73.9 d | 1''' | 4 | 4.43[c] td (4.6, 2.1) | 74.0 d | 1''' | 4 | 4.45[c] ddd (5.5, 4.6, 2.2) | 74.1 d |
| 4 | 2.82 ad (16.2, 4.6)<br>2.74 dd (16.2, 5.5) | 25.7 t | 2 | | 2.80 d (4.6) | 26.2 t | 2 | | 2.79 dd (16.5, 4.6)<br>2.72 dd (16.5, 5.5) | 25.5 t |
| 4a | — | 101.2 s | 3, 6 | 4 | — | 101.3 s | 3, 6 | 4 | — | 101.1 s |
| 5 | — | 157.7 s | 4 | 6 | — | 157.8 s | 4 | 6 | — | 158.6 s |
| 6 | 6.00 s | 97.2 d | | | 6.00 s | 97.3 d | | | 5.92 d (2.2) | 97.2 d |
| 7 | — | 157.1 s | 1'' | 6 | — | 157.1 s | 1'' | 6 | — | 158.7 s |
| 8 | — | 108.5 s | 1'', 2'', 6 | | — | 108.5 s | 1'', 2'', 6 | | 5.89 d (2.2) | 96.4 d |
| 8a | — | 156.1 s | 1'', 2, 4 | | — | 156.4 s | 1'', 2, 4 | | — | 157.9 s |
| 1' | — | 132.3 s | 3, 5' | 2, 2' | — | 132.3 s | 3, 5' | 2, 2' | — | 132.4 s |
| 2' | 7.04[d] d (1.8) | 117.0 d | 2, 6' | | 7.04[d] d (1.8) | 116.9 d | 2, 6' | | 7.05[d] d (2.1) | 117.0 d |
| 3' | — | 146.3 s | 5' | 2' | — | 146.2 s | 5' | 2' | — | 146.2 s |
| 4' | — | 146.6 s | 2', 6' | 5' | — | 146.5 s | 2', 6' | 5' | — | 146.5 s |
| 5' | 6.71 d (8.2) | 116.5 d | | | 6.71 d (8.2) | 116.5 d | | | 6.69 d (8.2) | 116.3 d |
| 6' | 6.80[d] dd (8.2, 1.8) | 121.1 d | 2, 2' | | 6.81[d] dd (8.2, 1.8) | 120.9 d | 2, 2' | | 6.80[d] dd (8.2, 2.1) | 121.1 d |
| 1'' | 5.39 dd (8.6, 5.5) | 51.1 d | 3'' | 2'' | 5.41 dd (9.2, 5.5) | 51.2 d | 3'' | 2'' | — | — |
| 2'' | 2.37 m<br>2.33 m | 27.7 t | | 1'', 3'' | 2.38 m<br>2.28 m | 27.9 t | | 1'', 3'' | — | — |
| 3'' | 2.47 m<br>2.28 m | 33.0 t | | 2'' | 2.46 m<br>2.30 m | 33.0 t | | 2'' | — | — |
| 4'' | — | 182.3 s | 1'', 2'' | 3'' | — | 182.1 s | 1'', 2'' | 3'' | — | — |
| 1''' | 4.75[c] d (7.9) | 101.2 d | 3, 3''', 5''' | 2''' | 4.72[c] d (7.9) | 100.9 d | 3, 3''', 5''' | 2''' | 4.76[c] d (7.9) | 100.9 d |
| 2''' | 3.27 dd (7.9, 2.9) | 73.0 d | | 3''' | 3.26 dd (7.9, 2.9) | 73.1 d | | 3''' | 3.27 dd (7.9, 2.9) | 73.0 d |
| 3''' | 4.01 t (2.9) | 73.7 d | 1''', 5''' | 2''' | 3.99 t (2.9) | 73.7 d | 1''', 5''' | 2''' | 4.00 t (2.9) | 73.6 d |
| 4''' | 3.46 dd (9.2, 2.9) | 69.7 d | 6''' | 3''', 5''' | 3.43 dd (9.5, 2.9) | 69.7 d | 6''' | 3''', 5''' | 3.44 dd (9.5, 2.9) | 69.7 d |
| 5''' | 3.63 ddd (9.2, 5.2, 4.3) | 76.1 d | 3''' | 4''', 6''' | 3.59 ddd (9.5, 5.5, 4.3) | 76.0 d | 3''' | 4''', 6''' | 3.63 ddd (9.5, 6.0, 2.0) | 76.0 d |
| 6''' | 3.80 dd (13.7, 4.3)<br>3.62 dd (13.7, 5.2) | 64.0 t | 4''' | | 3.76 dd (13.7, 4.3)<br>3.57 dd (13.7, 5.5) | 64.0 t | 4''' | | 3.79 dd (12.5, 2.0)<br>3.60 dd (12.5, 6.0) | 64.0 t |

a) Signal assignments are based on the analyses of the $^1$H-$^1$H COSY, HMQC, and HMBC spectra.

b) $^3J_{CH}$ and $^2J_{CH}$ indicate the protons long-range-coupled with carbons through three and two bonds, respectively, which were observed in the HMBC spectra.

c) NOE was observed between each other. d) Long-range coupling was observed with 2-H in the $^1$H-$^1$H COSY.

1). Among three oxygenated ring-A carbons at δ156-158 region,[6] the one at δ156.1 (C-8a) showed long-range correlations with the protons at δ2.74, 2.82 (4-$H_2$), and 5.13 (2-H), while the carbons at δ157.7 (C-5) and at δ157.1 (C-7) showed correlations with the protons at δ2.74 and 2.82 (4-$H_2$) and an isolated aromatic proton at δ6.00 (6-H) and with the proton at δ6.00 (6-H), respectively. Therefore, these carbons were unambiguously assigned to C-8a, C-5, and C-7, respectively, and the isolated aromatic proton at δ6.00 was assigned to 6-H. Further, the carbons C-7 (δ157.1) and C-8a (δ156.1) both showed long-range correlation with the methine proton (δ5.39, 1''-H) of the pyrrolidinone group (Fig. 1). Also some other significant long-range correlations observed are shown by arrows in the formula in Fig. 1.

From these observations, davallioside A was determined to be 8-(2-pyrrolidinone-5-yl)-epicatechin-3-O-β-D-allopyranoside (**1**).

Davallioside B (**2**), amorphous solid, $[\alpha]_D^{23}$ -114.6° (MeOH), has the molecular formula $C_{25}H_{29}NO_{12}$ (FAB-MS m/z: 536 $[M+H]^+$, 558 $[M+Na]^+$). Its UV and IR spectra were very similar to those of **1**. The $^1$H- and $^{13}$C-NMR spectra (Table I) also closely resembled those of **1**, and the extensive analysis with the aid of $^1$H-$^1$H COSY, HMQC, and HMBC indicated that **2** should be a diastereoisomer of **1**.

In the CD spectra, **1** and **2** both showed a negative Cotton effect at around 280 nm ($^1L_b$ transition),[7] showing that both compounds have the R-configuration at the C-2 position.[8] Thus, davallioside A and B are diastereoisomers at the C-1'' position of 8-(2-pyrrolidinone-5-yl)-(-)-epicatechin-3-O-β-D-allopyranoside shown as **1** and **2**. The absolute configuration of **1** and **2** and their biological activity are currently under investi-

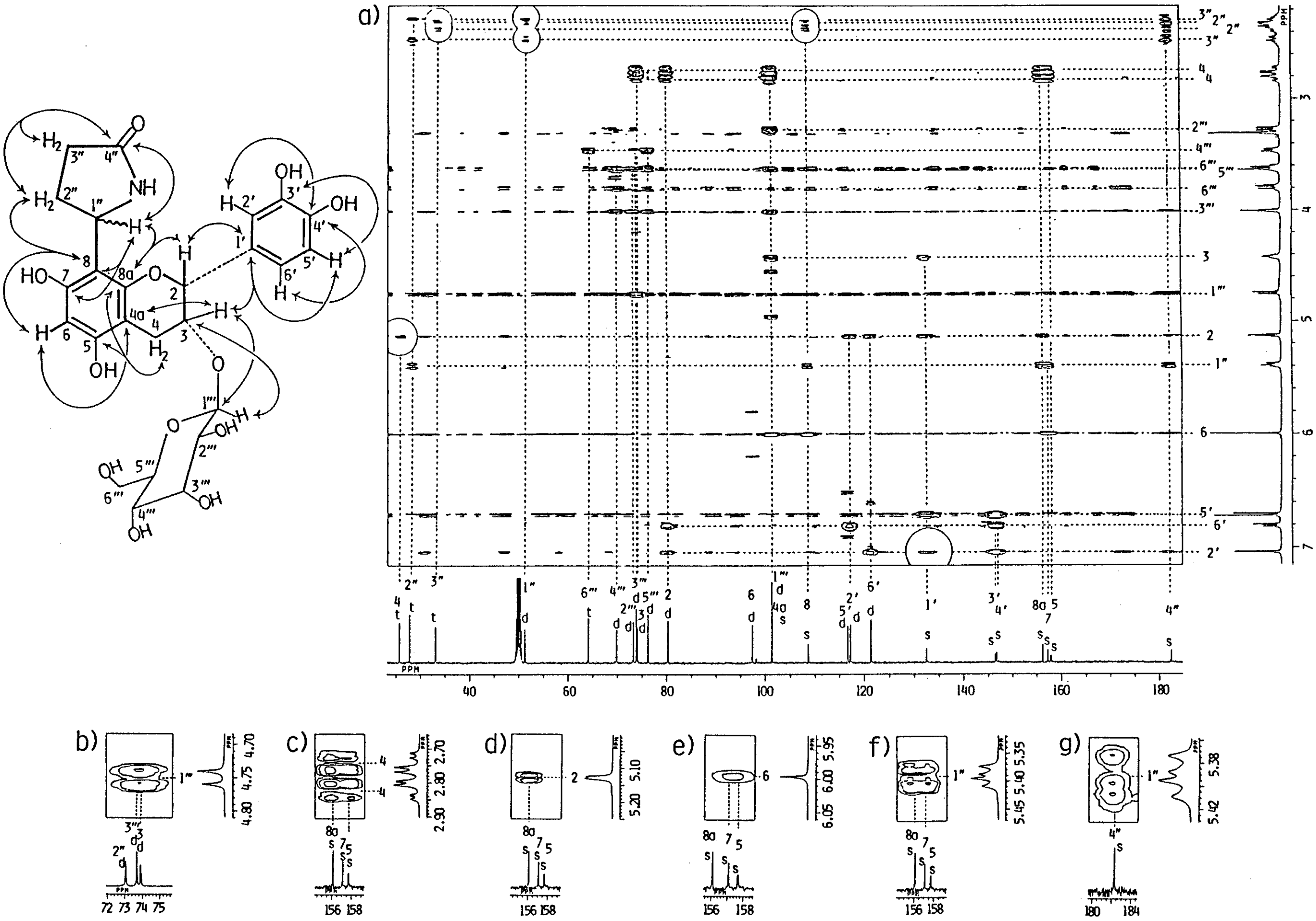


Fig. 1. HMBC Spectrum of **1** in Methanol-$d_4$ (Sample: 24.8 mg, Long-range $J_{CH}$=8.3 Hz, 12 h run)
a) Whole region. b), c), d), e) Cross peaks of 1‴-H, 4-H, 2-H and 6-H, respectively. f), g) Cross peaks of 1″-H. Open circles indicate significant but weak peaks at this threshold level.

gation. Our present result provided the first example of flavan-3-ol derivatives with a γ-lactam substituent.9)

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