DAVALLIOSIDE A AND B, NOVEL FLAVAN-3-OL DERIVATIVES WITH A γ -LACTAM, FROM THE RHIZOMES OF *DAVALLIA MARIESII* MOORE¹⁾

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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.

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-allopyranoside⁴⁾ (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 F₂₅₄ plates; EtOAc-EtOH-H₂O (10:2:1)] followed by HPLC separation [column, TSK-GEL ODS-120A, 21.5 mm x 35 cm; solvent, MeOH-H₂O (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 $[\alpha]_D^{25} +10.2^\circ$ (MeOH) and its molecular formula was determined to be C₂₅H₂₉NO₁₂ 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 ¹³C-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 CHCH₂CH₂ residue (δ_H 5.39, 2.28, 2.47, 2.33, and 2.37; δ_C 51.1, 33.0, and 27.7) and a carbonyl group (δ_C 182.3).

Detailed analysis of the ¹H-¹H COSY and HMQC⁵⁾ 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 HMBC⁶⁾ spectrum (Fig.

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

Compd. Position	1			2			3	
	δ_{H} (J Hz)	δ_{C} ($^3\text{J}_{\text{CH}}$ ^1H L.r. coupled ^{b)} $^2\text{J}_{\text{CH}}$)		δ_{H} (J Hz)	δ_{C} ($^3\text{J}_{\text{CH}}$ ^1H L.r. coupled ^{b)} $^2\text{J}_{\text{CH}}$)		δ_{H} (J Hz)	δ_{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 dd (16.2, 4.6) 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) 2.72 dd (16.5, 5.5)	25.5 t
4a	—	101.2 s	3, 6	—	101.3 s	3, 6	—	101.1 s
5	—	157.7 s	4	—	157.8 s	4	—	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''	—	157.1 s	1''	—	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'	—	132.3 s	3, 5'	—	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'	—	146.2 s	5'	—	146.2 s
4'	—	146.6 s	2', 6'	—	146.5 s	2', 6'	—	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''	5.41 dd (9.2, 5.5)	51.2 d	3''	—	—
2''	2.37 m 2.33 m	27.7 t	1'', 3''	2.38 m 2.28 m	27.9 t	1'', 3''	—	—
3''	2.47 m 2.28 m	33.0 t	2''	2.46 m 2.30 m	33.0 t	2''	—	—
4''	—	182.3 s	1'', 2''	—	182.1 s	1'', 2''	—	—
1'''	4.75 ^{c)} d (7.9)	101.2 d	3, 3''', 5'''	4.72 ^{c)} d (7.9)	100.9 d	3, 3''', 5'''	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'''	3.99 t (2.9)	73.7 d	1''', 5'''	4.00 t (2.9)	73.6 d
4'''	3.46 dd (9.2, 2.9)	69.7 d	6'''	3.43 dd (9.5, 2.9)	69.7 d	6'''	3.44 dd (9.5, 2.9)	69.7 d
5'''	3.63 ddd (9.2, 5.2, 4.3)	76.1 d	3'''	3.59 ddd (9.5, 5.5, 4.3)	76.0 d	3'''	3.63 ddd (9.5, 6.0, 2.0)	76.0 d
6'''	3.80 dd (13.7, 4.3) 3.62 dd (13.7, 5.2)	64.0 t	4'''	3.76 dd (13.7, 4.3) 3.57 dd (13.7, 5.5)	64.0 t	4'''	3.79 dd (12.5, 2.0) 3.60 dd (12.5, 6.0)	64.0 t

a) Signal assignments are based on the analyses of the ^1H - ^1H COSY, HMQC, and HMBC spectra.b) $^3\text{J}_{\text{CH}}$ and $^2\text{J}_{\text{CH}}$ 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 ^1H - ^1H COSY.

1). Among three oxygenated ring-A carbons at δ 156-158 region,⁶⁾ the one at δ 156.1 (C-8a) showed long-range correlations with the protons at δ 2.74, 2.82 (4-H₂), and 5.13 (2-H), while the carbons at δ 157.7 (C-5) and at δ 157.1 (C-7) showed correlations with the protons at δ 2.74 and 2.82 (4-H₂) and an isolated aromatic proton at δ 6.00 (6-H) and with the proton at δ 6.00 (6-H), respectively. Therefore, these carbons were unambiguously assigned to C-8a, C-5, and C-7, respectively, and the isolated aromatic proton at δ 6.00 was assigned to 6-H. Further, the carbons C-7 (δ 157.1) and C-8a (δ 156.1) both showed long-range correlation with the methine proton (δ 5.39, 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, [α]_D²⁵-114.6° (MeOH), has the molecular formula C₂₅H₂₉NO₁₂ (FAB-MS m/z : 536 [M+H]⁺, 558 [M+Na]⁺). Its UV and IR spectra were very similar to those of **1**. The ^1H - and ^{13}C -NMR spectra (Table I) also closely resembled those of **1**, and the extensive analysis with the aid of ^1H - ^1H 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 ($^1\text{L}_b$ 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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