

A New Flavan-3-ol Glucoside from *Daphniphyllum oldhami*

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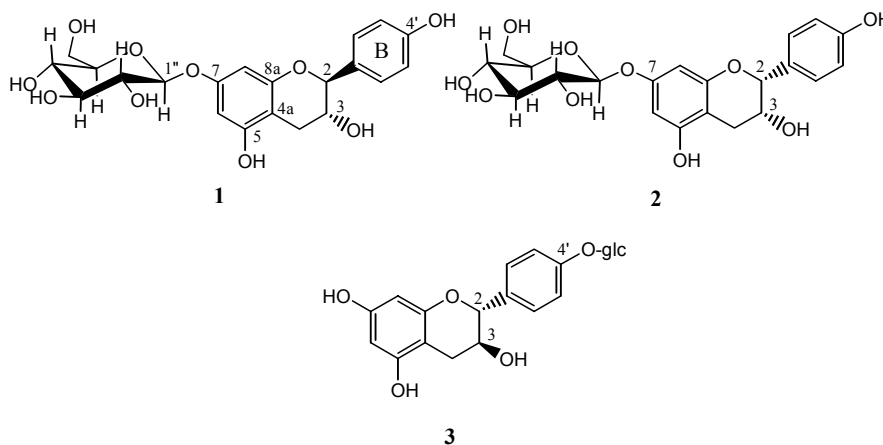
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Abstract: A new flavan-3-ol glucoside, (-)-afzelechin-7-*O*- β -D-glucopyranoside (**1**), has been isolated from the stem of *Daphniphyllum oldhami*. The structure of the new compound was elucidated on the basis of detailed spectroscopic analysis and comparison with related compounds.

Keywords: *Daphniphyllum oldhami*, flavan-3-ol, (-)-afzelechin-7-*O*- β -D-glucopyranoside.

In a previous paper¹, we reported the isolation and structural determination of two new triterpenoids from *Daphniphyllum oldhami*. Recently, on our continuing study on this plant for medicinal agents, a new flavan-3-ol glucoside, (-)-afzelechin-7-*O*- β -D-glucopyranoside (**1**), was isolated from the stem of the title plant. This paper deals with the isolation and structural elucidation of this new compound.

The usual work-up¹ of the EtOAc soluble fraction of the methanolic extract of the stem of *D. oldhami* yielded the new compound **1**.



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Compound **1**² was obtained as light-brown fine crystals from MeOH, mp 219-221 °C, $[\alpha]_D - 40.5$ [c 0.64, (Me)₂CO]. Its positive ESIMS spectrum displays a quasimolecular ion $[M+Na]^+$ at $m/z = 459$ corresponding to a molecular formula of C₂₁H₂₄O₁₀ for the compound, which was further confirmed by high resolution ESIMS spectrum {459.1270 $[M+Na]^+$, calcd. 459.1267}. Compound **1** was considered to contain a flavan-3-ol unit in the molecule, by interpretation of its spectral data and after the performance of appropriate colour tests (ferric chloride, anisaldehyde-sulphuric acid). In the ¹H NMR spectrum, signals due to a flavan-3-ol moiety, including a pair of two-proton doublets at δ 7.21 ($J = 8.5$ Hz) and 6.78 ($J = 8.5$ Hz) due to a ring-B *p*-hydroxylphenyl group, as well as an anomeric proton doublet at δ 4.81 ($J = 7.4$ Hz), were observed. In the ¹³C NMR spectrum (**Table 1**), 19 carbon signals were observed from a flavan-3-ol unit and a hexose sugar. Therefore, **1** was inferred as 5, 7, 4'-hydroxyl-flavan-3-ol bearing one hexose sugar. Analysis of ¹H-¹H COSY spectrum, from the anomeric proton (H-1''), the assignments of H-2'' (δ 3.12, m), H-3'' (δ 3.26, m), H-4'' (δ 3.30, m), H-5'' (δ 3.45, m), and H₂-6'' (δ 3.68, dd, $J = 12.0, 5.0; 3.87, dd, J = 12.0, 1.0$) could be made. The corresponding carbon signals were assigned as δ 102.2 (C-1''), 74.8 (C-2''), 78.0 (C-3''), 71.3 (C-4''), 77.9 (C-5''), and 62.5 (C-6''), respectively, by HMQC experiment. These spectral data suggested that the sugar was glucose, which was also confirmed by comparison on TLC with authentic sample after hydrolysis. The position of the glucose unit was concluded to be at C-7 from the ¹H-¹H NOESY spectrum of **1** which showed two correlation cross-peaks between the H-1'' and H-6 and H-8. The configuration of the anomeric carbon of D-glucose was determined to be β on the basis of the coupling constant ($J = 7.4$ Hz) of the anomeric proton signal in the ¹H NMR of **1**. In fact, The ¹H and ¹³C NMR spectra of **1** was very similar to those of 7-*O*- β -D-glucopyranosyl(-)-epiafzelechin (**2**)³, and also closely correlated with those of 4'-*O*- β -D-glucopyranosyl(+)-afzelechin (**3**)⁴. Careful comparison of NMR data of **1** with those of **2** (**Table 1**) and **3**⁴ revealed that the mainly difference between **1** and **2** was the splitting pattern of H-2 signal, where the former exhibited a large coupling constant ($J = 7.7$ Hz), the same as that of compound **3**, while the latter appeared as a broad singlet. This fact suggested that compound **1** possessed 2,3-*trans* stereochemistry (afzelechin-type)⁴. Furthermore, **1** exhibited a negative specific optical rotation [$- 40.5, c$ 0.64, (Me)₂CO], opposite to that of **3** [$+ 37, c$ 1.2, (Me)₂CO], suggesting that the absolute stereochemistry of **1** at C-2 and C-3 was enantiomeric with the corresponding chiral centers in compound **3**^{5,6}. Accordingly, **1** was characterized as (-)-afzelechin- 7-*O*- β -D-glucopyranoside.

Afzelechin-type flavan-3-ols are rare in nature. Compound **1** has not been isolated previously from natural source. The immune activities of compound **1** was tested, but it showed no significant bioactivity. Other bioassays for the compound are currently ongoing.

Table 1 ^1H and ^{13}C NMR data for compound **1** and comparison with those of **2**

No.	^1H (J in Hz)	1 ^a		2 ^b	
		δ ^{13}C	δ ^1H (J in Hz)	δ ^{13}C	δ ^1H (J in Hz)
2	4.61 (d, 7.7)	82.9 (d)	4.85 (br s)	79.7 (d)	
3	4.00 (m)	68.6 (d)	4.12 (m)	66.7 (d)	
4	2.53 (dd, 8.4, 16.4); 2.89 (dd, 5.5, 16.4)	28.9 (t)	2.60 (dd, 2.0, 16.0); 2.78 (dd, 3.0, 16.0)	29.1 (t)	
4a	-	103.7 (s)	-	102.9 (s)	
5	-	157.5 (s)	-	157.6 (s)	
6	6.20 (d, 2.3)	97.4 (d)	6.20 (d, 1.0)	97.5 (d)	
7	-	158.7 (s)	-	158.1 (s)	
8	6.14 (d, 2.3)	96.9 (d)	6.05 (d, 1.0)	97.0 (d)	
8a	-	156.9 (s)	-	157.0 (s)	
1'	-	131.3 (s)	-	131.1 (s)	
2', 6'	7.21 (d, 8.5)	129.6 (d)	7.22 (d, 8.0)	129.3 (d)	
3', 5'	6.78 (d, 8.5)	116.1 (d)	6.72 (d, 8.0)	115.8 (d)	
4'	-	158.4 (s)	-	157.7 (s)	
1''	4.81 (d, 7.4)	102.2 (d)	4.80 (d, 8.0)	101.9 (d)	
2''	3.30-3.40 (m)	74.8 (d)	3.30-3.50 (m)	74.4 (d)	
3''		78.0 (d)	3.30-3.50 (m)	77.5 (d)	
4''		71.3 (d)	3.30-3.50 (m)	71.0 (d)	
5''		77.9 (d)	3.30-3.50 (m)	77.4 (d)	
6''	3.68 (dd, 12.0, 5.0) 3.87 (dd, 12.0, 1.0)	62.5 (t)	3.60 (dd, 12.0, 5.0) 3.79 (dd, 12.0, 1.0)	62.3 (t)	

^a Bruker AMX 500 MHz; Measured in CD_3OD . Chemical shifts (δ) are expressed relative to TMS. Assignments were deduced by analysis of 1D and 2D spectra.

^b Measured in acetone- d_6 + D_2O .³

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References and Notes

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