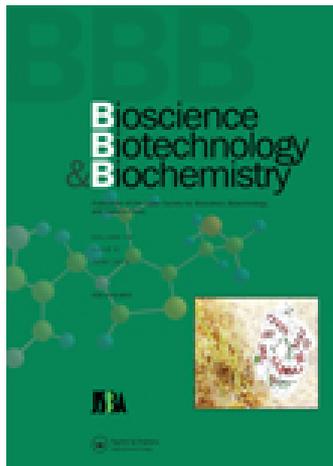


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Desmodianone H and uncinanone B, potential tyrosinase inhibitors obtained from *Lespedeza maximowiczii* by using bioactivity-guided isolation

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A new bioactive compound, namely desmodianone H (1), and another known compound uncinanone B (2) were first isolated using bioactivity-guided isolation from the leaves of *Lespedeza maximowiczii* and structures were elucidated by comprehensive analysis of their nuclear magnetic resonance and mass spectrometry data. Compounds 1 and 2 exhibited strong inhibitory effects on mushroom tyrosinase activity.

Key words: *Lespedeza maximowiczii*; desmodianone H; uncinanone B; tyrosinase inhibition activity

Lespedeza maximowiczii (Leguminosae) is a deciduous shrub, which is generally distributed in both Eastern North America and Eastern Asia.¹⁾ About 40 plant species belonging to the genus *Lespedeza* have been reported; these species show very diverse morphological characteristics.²⁾ Furthermore, many metabolites, especially isoflavanones,^{3–6)} phenolic compounds,⁷⁾ and chalcone⁸⁾ have been isolated from species belonging to this genus. *Lespedeza* plants have traditionally been considered to have antipyretic, anti-inflammatory, and diuretic activities.⁹⁾ In addition, compounds such as haginin A, dalbergioidin, and lespeflorin A–G that have been isolated from *Lespedeza cyrtobotrya* and *Lespedeza floribunda* are known to inhibit melanin synthesis *in vivo*.^{10–12)}

In our continuing research, we classified *L. maximowiczii* by significantly different phytochemicals between four species (*L. cyrtobotrya*, *L. bicolor*, *L. cuneata*, and *L. maximowiczii*), and *L. maximowiczii* showed the highest activity than other species (data not shown).¹³⁾ Herein, we report the isolation, structure elucidation, and tyrosinase inhibition activity of a new compound, desmodianone H (1), and a known compound, uncinanone B (2), from *L. maximowiczii*.

The leaves of *L. maximowiczii* were collected from Odo Mountain, Gajo Township, Guchang District, Gyeongsangnam-do, South Korea, in August 2011. The samples were identified by Joongku Lee et al., and a

voucher specimen was deposited at the Korea Research Institute of Bioscience and Biotechnology (KRIBB, Daejeon, Korea). Samples were dried under shade conditions and ground into a fine homogeneous powder by using a pulverizer. A sample of *Lespedeza* powder (120 g) was extracted with MeOH (3 × 600 mL, 2 d, room temperature, 200 rpm), and a methanol extract was obtained after evaporation of the solvent under vacuum, and it afforded 26% yield. The MeOH extract shows half-maximal inhibitory concentration value at 0.75 mg/mL. The extract was suspended in H₂O and partitioned with EtOAc (1/1, v/v), and the crude EtOAc fraction shows the IC₅₀ value at 0.12 mg/mL. The EtOAc fraction (2.98 g) was subjected to open column chromatography over Sephadex LH-20 resin (4 × 83 cm) by using isocratic elution with 80% MeOH to obtain 96 fractions.

These fractions were further purified with prep-HPLC using a YMC-Pack Pro C18 reversed-phase column (250 × 4.6 mm i.d. 5 μm; 5% CH₃CN in H₂O (v/v) – 100% CH₃CN = 95:5–0:100, v/v at a flow rate of 1 mL/min) equipped with a Hitachi photodiode array detector to obtain compound 1 (*t*_R 35 min, 7.8 mg) and compound 2 (*t*_R 38 min, 19.2 mg). One-dimensional and 2D NMR spectra were measured in methanol-*d*₄ (δ_H 3.30/δ_C 49.5) on a Bruker Avance 600 spectrometer (¹H: 600 MHz, ¹³C: 150 MHz). HRESIMS was obtained from Waters micromass Q-TOF premier with UPLC Acquity System (Waters, Milford, MA, USA) and Varian 500-MS ion-trap mass spectrometer (Palo Alto, CA, USA) was used for mass fragment data. Optical rotation data were recorded with Jasco P-1020 polarimeter (Manasquan, New Jersey, USA). All the steps were selected on the basis of the mushroom tyrosinase inhibition activity assay, which is a basic method to confirm the inhibition of melanin synthesis.^{14,15)}

The mushroom tyrosinase inhibitory activity was determined as described previously with some modification.¹¹⁾ The reaction mixture, consisted of 153 μL of 0.1 M sodium phosphate buffer (pH 6.5), 5 μL of sample dissolved in methanol, 5 μL of mushroom tyrosinase (2,500 unit/mL), and 36 μL of 1.5 mM

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L-tyrosine, was added to a 96 well plate. After the reaction mixture was incubated at 37 °C for 20 min, absorbance was measured at 490 nm with a microplate reader (Biotek, EL808, Seoul, Korea). The absorbance of the same mixture with the MeOH was used as control. Kojic acid was used as positive control. Each treatment was replicated three times. The percent inhibition of tyrosinase inhibition activity was calculated as follows:

$$\% \text{ Inhibition} = [(C_{20 \text{ min}} - C_{0 \text{ min}}) - (S_{20 \text{ min}} - S_{0 \text{ min}})] / (C_{20 \text{ min}} - C_{0 \text{ min}}) \times 100$$

whereas $C_{20 \text{ min}}$ is the absorbance of the control after 20 min, $C_{0 \text{ min}}$ is the absorbance of the control after 0 min, $S_{20 \text{ min}}$ is the absorbance of the experimental sample after 20 min and $S_{0 \text{ min}}$ is the absorbance of the experimental sample after 0 min.

Compound **1** was obtained as a pale yellow powder, and its molecular formula was determined as $C_{20}H_{18}O_5$ from its negative mode high resolution electron spray ionization mass spectroscopic data at m/z 337.1038 [$M-H$]⁻ (calcd. For $C_{20}H_{17}O_5$, 337.1076), which was compatible with the nuclear magnetic resonance data. The UV spectrum showed ketone absorption at λ_{max} 239.0 nm; in addition, the bands at 298 and 330 (sh) nm were suggestive of a flavanone skeleton. The specific optical rotation of **1** had plus (+) sign ($[\alpha]_D^{25} +21$ (c 0.07, MeOH)). The ^{13}C NMR spectrum of **1** (Table 1) displayed signals for 20 carbons, including one methyl group, one methylene, five methines, two methanetriyl groups, one carbonyl group, eight quaternary carbons, and two methylene bridges, as revealed by the

heteronuclear single quantum coherence (HSQC) experiment. Interpretation of the 1H - 1H correlation spectroscopy (COSY) data of **1** resulted in the identification of four proton spin-systems corresponding to H-2''-H-3'', H-5-H-6, H-2-H-3, and H-5'-H-6' units, and the remaining connections were established by the analysis of heteronuclear multiple-bond correlations (HMBC). The HMBC correlations from H-2'' to C-7 and from H-3'' to C-8 and C-2'' were all connected as a dihydrofuran ring. In addition, the correlations from H-5 to C-7 and from H-6 to C-7 and C-8 led to the connection of C-8 with C-7, C-6, and C-5, which completed a benzofuran unit. The gross structure of **1** was determined by 1H - 1H COSY, HSQC, and HMBC experiments, the key 1H - 1H COSY and HMBC correlations being given in Fig. 1. The structure of **1** is similar to that of desmodianone G, a dihydroxy furanose flavanone isolated from *Desmodium canum* (which has a similar ring core skeleton).¹⁶ However, desmodianone G has a hydroxylated quaternary carbon at C-5 and a quaternary carbon at C-6, with an extra methyl group. On the basis of the foregoing data, the structure of compound **1** was determined to be the new flavanone named desmodianone H.

The compound **2** was identified as uncinanone B by comparing the physicochemical and spectroscopic data with previously reported data.¹⁷ In the same way of the previous research, uncinanone B was detected as a diastereoisomers in our study. Uncinanone B has weak hydrogen bond between 5-OH and ketone group and this weak bond occurs at two signals with very small difference in NMR and UPLC-QTOF-MS data. To the

Table 1. 1H (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data (methanol- d_4) of desmodianone H (**1**) and uncinanone B (**2**).

Desmodianone H (1)			Uncinanone B (2)		
Position	δ_C	δ_H (J in Hz)	Position	δ_C	δ_H (J in Hz)
2	72.6	4.66 (m)	2	69.7	4.59 (m)
		4.47 (m)			4.43 (m)
3	49.1	4.14 (m)	3	45	3.90 (s)
4	195.1		4	196.7	
4a	117.6*		4a	101.6	
5	131.1	7.79 (d, 8.40)	5	159.5	
6	105.7	6.52 (d, 8.40)	6	106.5	
7	168.8		7	170.2	
8	114.8		8	91	5.95 (s)
8a	158.2		8a	164.4	
1'	117.6*		1'	115.5	
2'	159.6		2'	156.9	
3'	104.2	6.32 (s)	3'	105.7	6.32 (s)
4'	161.8		4'	156.9*	
5'	108.2	6.25 (m)	5'	108.8	6.25 (m)
6'	132.4	6.82 (t, 6.60)	6'	128.9	6.84 (t, 6.06)
2''	89.6	5.35 (m)	2''		
3''	32.6	2.95 (m)	3''		
		3.32 (m)			
4''			4''	30.3	2.85 (m)
					3.25 (m)
5''			5''	88.8	5.30 (t, 2.04)
6''	145.7		6''	143.2	
7''	113.1	5.09 (s)	7''	113.5	5.06 (s)
		4.91 (s)			4.90 (s)
8''	17.6	1.77 (s)	8''	17.1	1.75 (s)
2'-OH			2'-OH		
4'-OH			4'-OH		

*Assignment interchangeable.

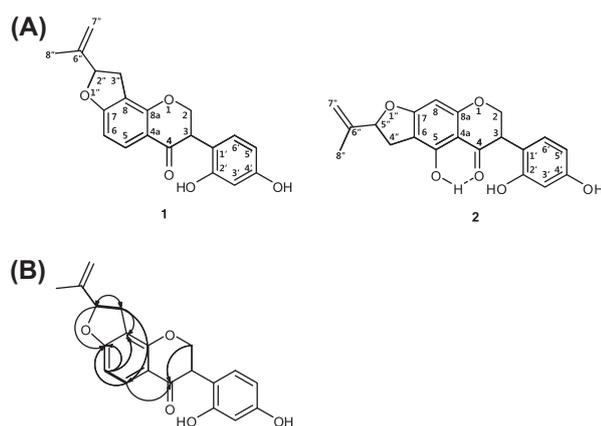


Fig. 1. Structure of compounds 1–2.

Notes: (A) Structures of compounds 1–2. (B) Key $^1\text{H} \rightarrow ^1\text{H}$ COSY (solid bond) and $^1\text{H} \rightarrow ^{13}\text{C}$ HMBC (arrow) correlations for 1.

Table 2. Mushroom tyrosinase inhibitory activity of the bioactive compounds.

Compound	IC ₅₀ (μM) ^a
Desmodianone H (1)	1.00 ± 0.25
Uncinanone B (2)	0.57 ± 0.01
Kojic acid ^b	84.0 ± 0.24

^aIC₅₀ value means half-maximal inhibitory concentration. Each value is expressed as the mean ± SD (*n* = 3).

^bPositive control.

best of our knowledge, this is the first report on the isolation of uncinanone B (2) from the leaves of *L. maximowiczii*.

The tyrosinase inhibition activity of the two isolated compounds, desmodianone H (1) and uncinanone B (2), was evaluated *in vitro* to calculate its IC₅₀ values. Compounds 1 and 2 exhibited remarkably higher anti-tyrosinase activity, (IC₅₀ values: 1.00 and 0.57 μM, respectively; Table 2) compared to kojic acid (IC₅₀ value, 84 μM), which was used as a positive control. Moreover, uncinanone B (2) showed higher potent inhibition rate than desmodianone H (1).

In conclusion, we isolated and identified the two compounds from *L. maximowiczii* leaves at the first time. Desmodianone H (1) is a novel compound that has tyrosinase inhibition activities. Together, uncinanone B (2) has higher anti-tyrosinase rate than desmodianone H. To our knowledge, this is the first report to investigate compounds from *L. maximowiczii* that inhibit the tyrosinase activities, suggesting that it could be a good candidate for skin-whitening agent.

Supplemental material

The supplemental material for this paper is available at <http://dx.doi.org/10.1080/09168451.2014.905180>.

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