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Extraction of Lignan from the Leaves of *Smallanthus sonchifolius* and its Antioxidant Activity

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A new lignan, smallanlignan B (**1**) together with seven known lignans (**2-8**) were isolated from the leaves of *S. sonchifolius*. Their structures were elucidated by the analysis of spectroscopic data. The antioxidant activity of smallanlignan B was also evaluated and it showed antioxidant activity with an IC_{50} value of 2.18 mg/mL.

Key Words: *Smallanthus sonchifolius*, Lignans, Antioxidant activity.

INTRODUCTION

Smallanthus sonchifolius is one of the members of asteraceae family. The tuber of *S. sonchifolius* is a welcome fruit because of its good taste and flavour. Sometimes, it is referred as the apple of the earth[1-3]. The leaves and roots of *S. sonchifolius* have received more and more attentions because of their biological potentialities. Previous phytochemical research on *S. sonchifolius* has revealed that flavones, terpenes, polyphenols, lignans, as well as polysaccharides are major principles isolated from this plant[4-15].

With the aim of continuing efforts to identify bioactive natural products from the plants, a chemical investigation on the leaves of *S. sonchifolius* indigenous to the Yuxi Prefecture of Yunnan Province in P.R. China was carried out. A new lignan, smallanlignan B(**1**) together with seven lignan, isoamericanol A(**2**)[16], epiwulignan A(**3**)[17], epischisandrone (**4**)[17], (-)-haplomyrfolin (**5**)[18], (-)-kusunokinin (**6**)[19], forsythialan B (**7**)[20] and 3,8,-(7 R, 8 S, 8' R)-7-ethoxy-3, 4:3', 4'-dimethylenedioxy- 8, 8'-lignan (**8**)[21]. Their structures (Fig. 1) were established by means of extensive NMR spectra and the antioxidant activity of compound (**1**) was also evaluated.

EXPERIMENTAL

Optical rotation was measured in Horiba SEPA-300 high sensitive polarimeter. IR spectra were obtained in KBr disc on a Bio-Rad wininfmred spectrophotometer. ESI-MS were measured on a VG auto spec-3000 MS spectrometer. ^1H, ^{13}C NMR and 2D NMR spectra were recorded on Bruker DRX-500 instruments with TMS as internal standard. On second separate used Agilent 1100 HPLC high performance liquid chromatography equipped with Zorbax-C_{18} (21.2 × 250 nm, 5.0 µm) column and DAD detector. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10-40 µm, Qingdao Marine Chemical Inc., P.R. China).

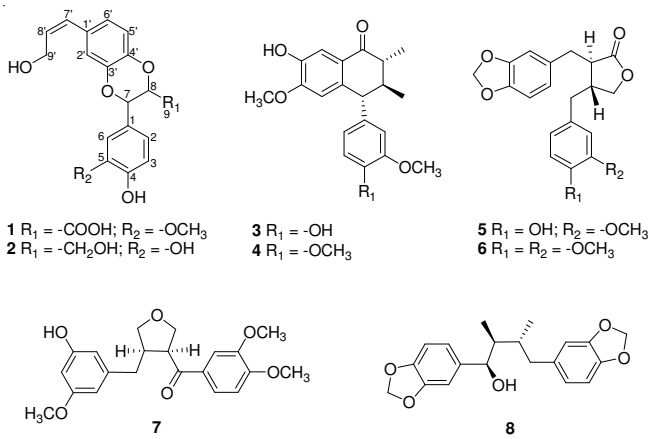

Fig. 1. Structure of lignans in *Smallanthus sonchifolius*

The leaves of *S. sonchifolius* were collected in Yuxi Prefecture of Yunnan Province, P.R. China, in September 2009 and were identified by Prof. S.F. WU. A voucher specimen (No. YNNI 09-20-09) was deposited in our laboratory.

Extraction and isolation: The air-dried and powdered leaves of *S. sonchifolius* (3.5 kg) were extracted four times with MeOH (4 × 3.5 L) at room temperature and filtered to yield a filtrate, which was condensed under the reduced pressure. The extract (158 g) was applied to Si gel (200 - 300 mesh) column chromatography eluting with a $CHCl_3$-MeOH gradient

system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A-F. The separation of fraction B (33.5 g) by Si gel column chromatography eluted with $CHCl_3$-$(Me)_2CO$ (20:1, 9:1, 8:2, 6:4, 5:5) yielded the mixtures B1-B5. Fraction B2 (5.2 g) was subjected to Si gel column chromatography using petroleum ether-$(Me)_2CO$ and preparative HPLC (65 % MeOH-H_2O, flow rate 12 mL/min) to give compounds **4** (25.6 mg), **6** (31.2 mg), **7** (22.5 mg) and **8** (28.4 mg). Fraction B3 (2.62 g) was subjected to Si gel column chromatography eluting with petroleum ether - $(Me)_2CO$ and then run on preparative HPLC (55 % MeOH-H_2O, flow rate 12 mL/min) to yield compounds **2** (15.4 mg), **3** (18.5 mg) and **5** (8.72 mg). Fraction B4 (1.85 g) was subjected to Si gel column chromatography eluting with petroleum ether - $(Me)_2CO$ and then run on preparative HPLC (40 % MeOH - H_2O, flow rate 12 mL/min) to give compound **1** (8.62 mg).

Antioxidant activity assay: Antioxidant activity was determined by the detection of the oxidative products with the 2',7'-dichlorofluorescin diacetate (DCFH) method reported previously[22].

RESULTS AND DISCUSSION

Smallanlignan B (**1**) was obtained as a white amorphous solid, showing $[\alpha]_D^{25.2}$ -26.3 (*c* 0.20, $CHCl_3$). The molecular formula of (**1**) was determined as $C_{19}H_{18}O_7$ by HR-ESI-MS (positive model), m/z 381.0955 $[M+Na]^+$ (calcd 381.0950). IR spectrum of (**1**) showed absorption at 3416 cm^{-1} for hydroxyl, 1782 cm^{-1} for carbonyl and 1610, 1595, 1518, 1460 cm^{-1} for aromatic groups. In the ^{13}C NMR spectrum of (**1**) (Table-1), 19 carbon signals were observed, including one carboxyl (δ_C 174.5), one methoxy (δ_C 61.8), one oxygen-bearing methylene (δ_C 61.8), two oxygen bearing methines (δ_C 85.6, 95.5), as well as eight aromatic methines and six quaternary aromatic carbons, which belong to two phenyls and one disubstituted double bond. The ^1H NMR signals at δ_H 4.31 (dd, *J* = 6.5, 1.8), δ_H 6.20 (dt, *J* = 15.8, 5.8), δ_H 6.42 (d, *J* = 11.2) indicated the presence of one (Z) allyl alcohol unit, which was also confirmed by ^1H-^1H COSY spectrum. In the HMBC spectrum, correlations between H-7, H-8 with C-9; H-8 with C-4'; H-7 with C-3', C-1, C-2, C-6 confirmed the structure fragment of benzodioxane. Further 2D NMR spectra data analysis (HSQC, COSY and HMBC) suggested that compound **1** possess a neolignan skeleton, which was composed of two phenylpropanoids units connected by a 1,4-dixoanering (Fig. 2).The ^1H and ^{13}C NMR spectra data of **1** were very similar to those of isoamericanol A[16], except for the presence of one additional methoxyl and one carbonyl in **1**, instead of one hydroxymethyl in isoamericanol A (Fig. 2). Thus, the structure of compound **1** was determined as shown and gives the name as smallanlignan B.

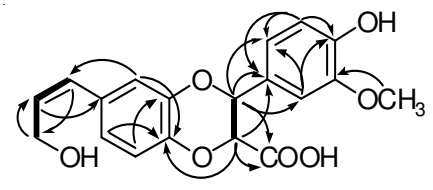

Fig. 2. Selected HMBC (↷) and ^1H-^1H COSY (–) of **1**

The antioxidant activity of smallanlignan B was determined by the detection of the oxidative products with the 2',7'-dichlorofluorescin diacetate (DCFH) method reported previously[22]. It shows antioxidant activity with an IC_{50} value of 2.18 μg/mL. Smallanlignan B shows high antioxidant activity.

TABLE-1
^1H NMR AND ^{13}C NMR DATA OF SMALLANLIGNAN B IN $CDCl_3$

No.	δ_C (mult)	δ_H (mult, *J*, Hz)
1	131.2 s	
2	111.5 d	6.94, d, *J* = 1.8
3	149.2 s	
4	146.2 s	
5	117.2 d	6.82, d, *J* = 8.6
6	120.5 d	6.74, dd, *J* = 8.2, 1.8
7	85.6 d	5.22, d, *J* = 8.2
8	95.5 d	5.46, d, *J* = 7.8
9	174.5 s	
1'	131.6 s	
2'	116.4 d	6.84 overlap
3'	144.2 s	
4'	142.9 s	
5'	116.9 d	6.84 overlap
6'	122.8 d	6.72, *J* = dd, 8.0, 1.8
7'	130.8 d	6.42, d, *J* = 11.2
8'	129.9 d	6.20, dt, *J* = 15.8, 5.8
9'	61.8 t	4.31, dd, *J* = 6.5, 1.8
-OMe	56.1 q	3.82 s

Smallanlignan B: White amorphous solid; $[\alpha]_D^{25.2}$ -26.3 (c 0.20, $CHCl_3$); UV λ_{max} ($CDCl_3$) nm (logε): 285 (3.65), 261 (3.92), 205 (4.45); IR (KBr, ν_{max}, cm^{-1}): 3416, 3224, 2927, 2880, 1782, 1610, 1595, 1518, 1481, 1375, 1268, 1106, 876, 824; ^1H- and ^{13}C NMR data are shown in Table-1. ESIMS (positive ion mode) m/z 381, HRESIMS (positive ion mode) m/z 381.0955 $[M+Na]^+$ (calcd. 381.0950 for $C_{19}H_{18}O_7$).

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