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Antiinflammatory Constituents from the Roots of *Smilax bockii* warb.

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From 70% ethanol extract of the roots of *Smilax bockii* warb., seven flavonoids, kaempferol (**1**), kaempferol-7-*O*-β-D-glucopyranoside (**2**), quercetin (**3**), isorhamnetin (**4**), (+)-dihydrokaempferol (**5**), engeletin (**6**), isoengeletin (**7**), and *n*-butyl-β-D-fructopyranoside (**8**), caffeic acid *n*-butyl ester (**9**) were isolated and identified by means of chemical and spectroscopic. Compounds **2**, **4**, and **6-9** were isolated for the first time from the roots of *S. bockii* and compounds **2**, **8**, and **9** were firstly isolated from the genus *Smilax*. In addition, using the SEAP (Secreted alkaline phosphatase) assay system, we investigated the *in vitro* anti-inflammatory activity of the 70% ethanol extract of the roots of *S. bockii*, which showed moderate activity in inhibiting TNF-α-induced NF-κB activation with an IC_{50} value of 166.6 μg/mL.

Key words: *Smilax bockii* warb., Flavonoids, *n*-Butyl-β-D-fructopyranoside, Caffeic acid *n*-butyl ester, Anti-inflammation activity

INTRODUCTION

Smilax. bockii warb. is a traditional Chinese medicine and belongs to the family Liliaceae, which is widely distributed in the south of China. It acts as an antirheumatic medicine and has anti-inflammation activity. In order to find the active constituents responsible for anti-inflammation, we investigated the constituents of the roots.

By means of chromatographic separation, nine compounds were isolated and identified on the basis of physical and chemical evidence and spectral methods as kaempferol (**1**), kaempferol-7-*O*-β-D-glucopyranoside (**2**), quercetin (**3**), isorhamnetin (**4**), (+)-dihydrokaempferol (**5**), engeletin (**6**), isoengeletin (**7**), *n*-butyl-β-D-fructopyranoside (**8**), caffeic acid *n*-butyl ester (**9**). Compounds **2**, **4**, and **6-9** were isolated for the first time from *S. bockii* and compounds **2**, **8**, and **9** were firstly isolated from the genus *Smilax*.

MATERIALS AND METHODS

General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Melting points were measured on a Yanaco-hot-stage without correction. All NMR spectra were recorded on a Brucker-ARX-300 spectrometer with TMS as an internal standard. UV spectra were recorded on a Shimadzu UV-260 UV-Vis spectrometer. IR spectra were measured on a Perkin-Elmer 2000 FT-2R spectrometer as KBr Pellets. EI-MS were measured on a VG-5050E mass spectrometer. Silica gel (200-300 mesh) for column chromatography and GF_{254} for TLC were produced by Qingdao Ocean Chemical Group Co. of China. Sephadex LH-20 was produced by Merck Co., Germany.

Plant materials

The roots of *S. bockii* were collected from a county of Hunan province, China, in April 2003. The plant material was identified and the voucher specimen (NO.20030406) was deposited in Research Department of Natural Medicine, Shenyang Pharmaceutical University, China.

Extraction and isolation

Dried roots (10 kg) of *S. bockii* were cut into small pieces and extracted three times with 70% ethanol. The ethanol extract (2 kg) was partitioned with $CHCl_3$, EtOAc and *n*-BuOH successively. The *n*-BuOH extract (200 g) was first subjected to column chromatography on silica gel gradiently eluted with $CHCl_3$:MeOH (from 100:0 to 100:70) to yield compound **1** (100:3, 45 mg), **2** (100:9, 350 mg), **6** (100:7, 60 mg), **7** (100:8, 420 mg), and **8**

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(100:5, 65 mg) as well as several fractions. Fraction 1 [$CHCl_3$:MeOH (100:4), 4.2 g] was further purified by sephadex LH-20 eluting with $CHCl_3$:MeOH to yield compound **3** (32 mg) and **4** (15 mg). Fraction 2 [$CHCl_3$:MeOH (100:2), 2.2 g] was rechromatographed on a silica gel column using petroleum etheracetone-ethyl acetate as eluent yielding compound **5** (28 mg). Fraction 3 [$CHCl_3$: MeOH (100:3), 2 g] was rechromatographed on a silica gel column eluted with petroleum ether-acetone-ethyl acetate to get compound **9** (32 mg).

Kaempferol (1)
Yellow powder; m.p. 274-276 °C; EI-MS m/z 286 $[M]^+$; UV λ_{max} nm: 266, 366 (MeOH); IR (KBr): 3380, 1655, 1608, 1600, 1503 cm^{-1}; ^1H -NMR data (see Table I).

Kaempferol-7-*O*-β-D-glucopyranoside (2)
Yellow powder; m.p. 267-268 °C; ^1H -NMR and ^{13}C-NMR data (see Table I and II).

Quercetin (3)
Yellow powder; m.p.>300 °C; ^1H-NMR and ^{13}C-NMR data (see Table I and II).

Isorhamnetin (4)
Yellow powder; m.p.>300 °C; ^1H-NMR and ^{13}C-NMR data (see Table I and II).

(+)-Dihydrokaempferol (5)
Yellow needles; m.p. 235-237 °C; EI-MS m/z 288 $[M]^+$; $[\alpha]_D^{23}$ +22.8*c* 1.32, MeOH; UV λ_{max} nm: 290, 329 sh; IR (KBr): 3366, 1660, 1605, 1550, 1500 cm^1; ^1H -NMR and ^{13}C-NMR data (see Table I and II).

Engeletin (6)
White powder; m.p. 169-171 °C; $[\alpha]_D^{20}$-14.2 (*c* 0.32, MeOH); EI-MS m/z 434 $[M]^+$, 288[M^+-rha]; UV λ_{max} nm: 292,336 sh; IR (KBr): 3520, 3350, 1638, 1592, 1513, 1465, 824 cm^1; ^1H-NMR and ^{13}C-NMR data (see Table I and II).

Table II. ^{13}C-NMR data for compounds **2-7** (75 MHz, DMSO-d_6)

No.	2	3	4	5	6	7
2	147.6	146.7	146.9	83.0	81.8	80.1
3	136.1	135.7	135.8	71.6	76.3	73.2
4	176.1	175.8	175.9	198.0	195.4	192.9
5	160.4	160.7	160.2	163.5	163.7	164.1
6	98.9	98.2	98.1	96.2	96.4	96.3
7	162.8	164.0	164.0	166.9	167.4	167.2
8	94.5	93.3	93.2	95.2	95.4	95.3
9	155.8	156.1	156.2	162.7	162.5	162.7
10	104.7	102.9	103.0	100.6	101.3	100.3
1'	121.6	121.9	122.1	127.7	126.8	125.9
2'	129.7	115.0	115.2	129.6	129.4	127.9
3'	115.5	145.0	147.1	115.1	115.5	114.9
4'	159.4	147.7	147.6	157.9	158.2	157.3
5'	115.5	115.6	115.7	115.1	115.5	114.9
6'	129.7	119.9	120.1	129.6	129.4	127.9
Glc-1"	100.0					
Rha-1"					100.6	98.66

Isoengeletin (7)
White powder; m.p. 294-296 °C; EI-MS m/z 434 $[M]^+$; UV λ_{max} nm: 295, 335 sh; IR (KBr): 3510, 3250, 1625, 1520, 1470, 840 cm^{-1}; ^1H-NMR and ^{13}C-NMR data (see Table I and II).

***n*-Butyl-β-D-fructopyranoside**
White needles; m.p. 152-153 °C; ^1H-NMR (300 MHz, C_5D_5N) δ: 0.78 (3H, t, H-4), 1.30 (2H, m, H-3), 1.51 (2H, m, H-2), 3.68 (2H, m, H-1), 4.07 (1H, d, *J* = 11.9 Hz, H-1'), 3.98 (1H, d, *J* = 11.9 Hz, H-1'), 4.88 (1H, d, *J* = 9.8 Hz, H-3'), 4.51 (1H, dd, *J* = 9.8, 3.4 Hz, H-4'), 4.35 (1H, d, *J* = 3.4 Hz, H-5'), 4.18 (1H, br d, *J* = 11.3 Hz, H-6'), 4.34 (1H, br d, *J* = 11.3 Hz, H-6'); ^{13}C-NMR (75 MHz, C_5D_5N) δ: 14.2 (C-4), 19.9 (C-3), 32.7 (C-2), 60.6 (C-1), 64.3 (C-1'), 101.2 (C-2'), 70.6 (C-3'), 72.3 (C-4'), 71.3 (C-5'), 65.0 (C-6').

Caffeic acid *n*-butyl ester
White needles; m.p. 111-113 °C; EI-MS m/z 236 $[M]^+$; IR(KBr): 3490, 3320, 1683, 1600, 1518, 1465, 758; ^1H-

Table I. ^1H-NMR data for compounds **1-7** (300 MHz, DMSO-d_6)

NO.	1	2	3	4	5	6	7
2					5.04(d, 11.0)	5.30(d, 10.4)	5.60(br s)
3					4.60(d, 11.0)	4.76(d, 10.4)	4.16(br s)
6	6.19(d, 2.0)	6.44(d, 1.7)	6.18(d, 2.1)	6.20(d, 1.7)	5.85(d, 2.0)	5.90(d, 2.0)	5.92(d, 2.0)
8	6.43(d, 2.0)	6.81(d, 1.7)	6.40(d, 2.1)	6.48(d, 1.7)	5.91(d, 2.0)	5.93(d, 2.0)	5.94(d, 2.0)
2'	8.04(d, 8.7)	8.10(d, 8.8)	7.67(d, 2.0)	7.76(d, 1.6)	7.31(d, 8.5)	7.34(d, 8.5)	7.24(d, 8.3)
3'	6.93(d, 8.7)	6.94(d, 8.8)			6.78(d, 8.5)	6.80(d, 8.5)	6.74(d, 8.3)
5'	6.93(d, 8.7)	6.94(d, 8.8)	6.87(d, 8.5)	6.94(d, 8.5)	6.78(d, 8.5)	6.80(d, 8.5)	6.74(d, 8.3)
6'	8.04(d, 8.7)	8.10(d, 8.8)	7.53(dd,8.5,2.0)	7.68(dd,8.5,1.6)	7.31(d, 8.5)	7.34(d, 8.5)	7.24(d, 8.3)
Glc-1"		5.42(d, 7.2)					
Rha-1"						4.50(d, 1.3)	4.78(d, 1.5)

NMR (300 MHz, DMSO-d_6) δ: 0.91 (3H, t, H-1'), 1.37 (2H, m, H-2'), 1.60 (2H, m, H-3'), 4.11 (2H, m, H-4'), 7.47 (1H, d, J = 15.9 Hz), 6.26 (1H, d, J = 15.9 Hz), 7.03 (1H, br.s , H-2), 7.01 (1H, dd, J = 8.1, 1.8 H_Z, H-6), 6.75 (1H, d, J = 8.1 H_Z, H-5), 9.51 (2H, br.s, 3,4-OH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 13.8 (C-4'), 18.8 (C-3'), 30.5 (C-2'), 63.5(C-1'), 166.8(C=O), 148.5 (C-7), 115.0 (C-8), 125.6 (C-1), 114.1 (C-2), 145.7 (C-3), 145.2 (C-4), 115.8 (C-5), 121.5 (C-6).

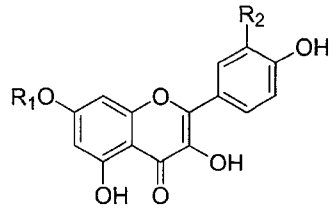
RESULTS AND DISCUSSION

The 70% ethanol extract of the roots of *S. bockii* was partitioned with $CHCl_3$, EtOAc and *n*-BuOH successively. The *n*-BuOH extract was separated by column chromatography to yield the seven known flavonoids **1-7** and *n*-butyl-β-D-fructopyranoside (**8**), caffeic acid *n*-butyl ester (**9**).

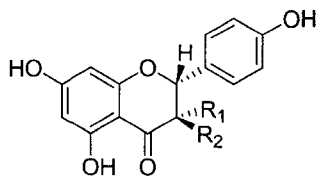
By analysis of the NMR data and the comparison of the physical and spectral data with compounds in literatures, compounds **1**, **3**, **4**, and **5** were identified as kaempferol (Qu *et al.*, 1995), quercetin (Markham *et al.*, 1978), isorhamnetin (Qu *et al.*, 1995), (+)-dihydrokaempferol (Ding *et al.*, 1997), respectively.

Compound **2** was isolated as a yellow powder and its melting point is 267-268 °C. The ^{1}H-NMR spectrum suggested that it had a kaempferol moiety. The signals at δ 8.10 (d, J = 8.8 Hz) and 6.94 (d, J = 8.8 Hz) are characteristic for the B ring, as well as δ 6.44 (d, J = 1.7 Hz) and 6.81 (d, J = 1.7 Hz) for the A ring. In addition it contained a sugar moiety from the ^{1}H-NMR and ^{13}C-NMR. The signal of the anomeric proton appeared at δ 5.42 (d, J = 7.2 Hz) with characteristic coupling constant of β-configuration. In particular, by the chemical shift of the six carbons at δ 100.0 (C-1''), 77.2-60.7 (C-2''-6'') in the ^{13}C-NMR spectrum, the sugar was confirmed to be β-D-glucose. The down field shifted signals of H-6 and H-8 compared with those of kaempferol suggested 7- hydroxyl bored β-D-glucose. On the basis of these data and by comparison with literature values (Li *et al.*, 2004), compound **2** was identified as kaempferol-7-*O*-β-D-glucopyranoside.

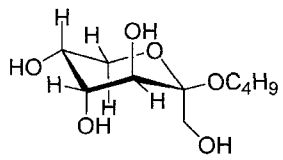
Compound **6** was obtained as white needles and its melting point is 169-171°C. Its molecular formula was established as $C_{21}H_{22}O_{10}$ by EI-MS (m/z 434[M]$^+$). The UV spectroscopy showed the presence of a flavanonol at 292, 336 nm. IR absorptions suggested the presence of a hydroxy group (3350 cm^{-1}), an α,β-unsaturated carbonyl group (1638 cm^{-1}), and an aromatic ring (1592, 1513, and 1465 cm^{-1}). The ^{1}H-NMR spectrum of **6** was similar with that of (+)-dihydrokaempferol, except that it contained a sugar moiety. Signals at δ 7.34 (2H, d, J = 8.5 Hz), 6.80 (2H, d, J = 8.5 Hz) are characteristic for the B ring, along with two meta-coupled doublets at δ 5.90 (1H, d, J = 2.0 Hz) and δ 5.93 (1H, d, J = 2.0 Hz) for the A ring; the coupling constant between proton δ 5.30 (1H, d, J = 10.4 Hz, H-2) and 4.76 (1H, d, J = 10.4 Hz, H-3) in the ^{1}H-NMR spectrum shows *trans*-configuration of H-2 and H-3. In addition, an anomeric proton at δ 4.50 (d, J = 1.3 Hz) and a methyl group δ 1.06 (3H, d, J = 6.0 Hz) in the ^{1}H-NMR spectrum, as well as ^{13}C-NMR signals at δ 100.6 (C-1) and 18.1 (C-6) suggested the presence of a rhamnose moiety. Based on its coupling constant of anomeric proton (J = 1.3 Hz) and compared the ^{13}C-NMR data with 1-



	R_1	R_2
1	H	H
2	Glc	H
3	H	OH
4	H	OCH_3



	R_1	R_2
5	H	OH
6	H	*O*-Rha
7	*O*-Rha	H



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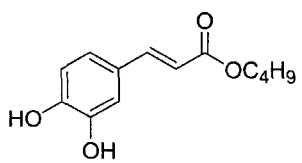

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Fig. 1. Structures of compounds **1-9** from *Smilax bockii* warb.

methyl-α-L-rhamnopyranose, an α-rhamnose was confirmed. In the case of a β-rhamnose, the coupling constant normally appears at approximately 4.2 Hz (Yue *et al.*, 2001). Compared with (+)-dihydrokaempferol, the chemical shift of C-3 in ^{13}C-NMR spectrum was shifted downfield (+4.8 ppm). Therefore, the rhamnose was attached to the C-3 position. On the basis of these data, compound **6** could be assigned as engeletin by direct comparson of NMR spectral data with those reported in the literature (Dulce *et al.*, 1997).

Compound **7** was obtained as white needles and its melting point is 294-296 °C. The ^{1}H-NMR and ^{13}C-NMR spectrum were similar to those of **6**, except for the coupling constant. δ 5.60 (1H, br.s) and 4.16 (1H, br.s) for the C ring showed *cis*-configuration of H-2 and H-3. On the basis of these data, compound **7** can be assigned as isoengeletin (Dulce *et al.*, 1997).

Compound **8** was obtained as white needles and its melting point is 152-153 °C; In the ^{1}H-NMR spectrum, δ 0.78 (3H, t, H-4), 1.30 (2H, m, H-3), 1.51 (2H, m, H-2), 3.68 (2H, m, H-1) are the *n*-butyl signals; in addition, the signals at δ 4.07 (1H, d, J = 11.9 Hz, H-1'), 3.98 (1H, d, J = 11.9 Hz, H-1'); 4.88 (1H, d, J = 9.8 Hz, H-3'), 4.51 (1H, dd, J = 9.8, 3.4 Hz, H-4'), 4.35 (1H, d, J = 3.4 Hz, H-5'), 4.18 (1H, br d, J = 11.3 Hz, H-6'), 4.34 (1H, br d, J = 11.3 Hz, H-6') were the signals of fructose. In particular, by the chemical shift of the carbons at δ 64.3 (C-1'), 101.2 (C-2'), 70.6 (C-3'), 72.3 (C-4'), 71.3 (C-5'), 65.0 (C-6') in the ^{13}C-NMR spectrum, the sugar was identified as fructose. On the basis of these data, compound **8** could be assigned as *n*-butyl-β-D-fructopyranoside (Zhang *et al.*, 1996).

Compound **9** was obtained as white needles and its melting point is 152-153 °C; Its molecular formula was established as $C_{13}H_{16}O_4$ by EI-MS (m/z 236 $[M]^+$). IR absorptions suggested the presence of a hydroxy group (3320 cm^{-1}), an α, β-unsaturated carbonyl group (1683 cm^{-1}), and an aromatic ring (1600, 1518, and 1465 cm^{-1}). The ^{1}H-NMR spectrum showed a methyl group at δ 0.91 (3H, t), two methylene groups at δ 1.37 (2H, m), 1.60 (2H, m), and a methenyl group at 4.11 (2H, m), which were assigned to *n*-butyl signals; signals at δ 7.03 (1H, br s), 7.01 (1H, dd, J = 8.1, 1.8 Hz), 6.75 (1H, d, J = 8.1 Hz) are assignable to three aromatic protons of the ABX system. In addition, δ 7.47 (1H, d, J = 15.9 Hz) and 6.26 (1H, d, J = 15.9 Hz) were two olefinic protons in *trans*-configuration. In the ^{13}C-NMR spectrum, δ 166.8 suggested the presence of α, β-unsaturated carbonyl. On the basis of these data, compound **9** could be assigned as caffeic acid *n*-butyl ester (Hu *et al.*, 1997).

Among these isolated compounds, compound **8** were reported as a specific inhibitor of Ig-E antibody formation (Haraguchi *et al.*, 1982); and compound **9** showed very potent inhibitory activity towards 5-lipoxygenase, its IC_{50} value being less than one-fourteenth of that of caffeic acid (Masanori *et al.*, 1989).

The extracts from the roots of *S. bockii* were examined for their dose-response effects against the activity of TNF-α-induced NF-κB activation in murine macrophage RAW 264.7 cells transfected with a NF-κB-mediated reporter gene construct (Koo *et al.*, 2001). The 70% ethanol extract showed moderate inhibitory activity against TNF-α-induced NF-κB activation with an IC_{50} value of 166.6 μg/mL. We further investigated the activity of $CHCl_3$, EtOAc and *n*-BuOH parts, the *n*-BuOH part showed relevant inhibitory activity with the IC_{50} value of 44.8 μg/mL, while $CHCl_3$, EtOAc parts were nearly inactive. These results showed that the active part of inhibiting TNF-α-induced NF-κB activation was *n*-BuOH part. According to *in vitro* experiments result, we will further investigate the constituents of *n*-BuOH part and study the anti-inflammatory activities of the isolated compounds to find the active compounds.

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