Coumarinolignans Isolated from the Seeds of *Brucea javanica*

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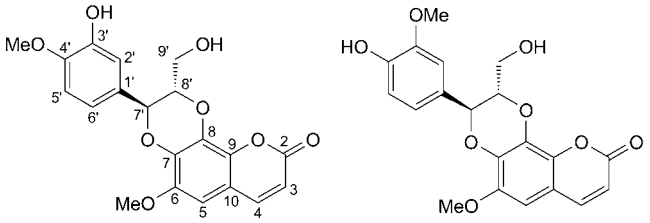
A new coumarinolignan, cleomiscosin E (**1**), together with the known compound cleomiscosin A (**2**), has been isolated from the seeds of *Brucea javanica* (L.) MERR. Their structures were assigned on the basis of spectral studies. These two compounds exhibited potent anti-inflammatory activities by inhibiting the nitric oxide (NO) production in lipopolysaccharide (LPS)-activated RAW264.7 macrophages.

Introduction. – *Brucea javanica* (L.) MERR. is a shrub that is widely distributed in Southeast Asia and northern Australia. Its seeds have been used for the treatment of inflammation, dysentery, malaria, and cancer [1][2]. Quassinoids [3–5] and coumarinolignans [6] have been isolated from this plant in recent decades. Our study on the chemical constituents from *Brucea javanica* led to the isolation of a new coumarinolignan cleomiscosin E (**1**) and the known compound cleomiscosin A (**2**) (*Fig. 1*). Herein, we describe the isolation, structure elucidation, and anti-inflammatory activities of the two compounds.



1 **2**

Fig. 1. *Structures of compounds* **1** *and* **2** *isolated from the seeds of* Brucea javanica

Results and Discussion. – Cleomiscosin E (**1**) was isolated as a pale-yellow powder ($[\alpha]_D^{21} = -2.3$ ($c = 0.08$ MeOH)). The $[M+H]^+$ ion peak at m/z 387.1082 (calc. 387.1080) in the high-resolution (HR) ESI-MS corresponded to the molecular formula $C_{20}H_{18}O_8$. This formula could also be confirmed through ^{1}H- and ^{13}C-NMR, as well as DEPT NMR spectra (*Table 1*). The UV maxima at 324 and 233 nm, and IR absorption at 1721 cm^{-1} suggested that **1** had characteristics of a coumarin derivative. This was

supported by the C=C-bond signals at δ(H) 6.34 (H–C(3)) and 7.97 (H–C(4)) with coupling constants of 9.6 Hz in the ^{1}H-NMR spectrum. Additional features of **1** that deduced from the chemical shift and splitting pattern of its ^{1}H-NMR signals in (D_6)DMSO were the presence of one OH group (δ(H) 9.24; signal disappeared by the addition of D_2O), one 1,3,4-trisubstituted phenyl group (δ(H) 7.03 (*d*, $J = 1.2$, H–C(2′)), 6.80 (*d*, $J = 8.0$, H–C(5′)), and 6.87 (*dd*, $J = 8.0, 1.2$, H–C(6′))), one aromatic CH group (δ(H) 6.92 (*s*, H–C(5))), two aromatic MeO groups δ(H) 3.79 (*s*, MeO–C(4′)) and 3.78 (*s*, MeO–C(6))), and a fragment with three O-bearing C-atoms (–CH(O)CH(O)CH_2OH; δ(H) 4.99 (*d*, $J = 7.6$, H–C(7′)), 4.30–4.36 (*m*, H–C(8′)), 3.63 (br. *d*, $J = 12.4$, H–C(9′)) and 3.37–3.41 (*m*, H–C(9′))). The above spectral data were closely related to those of the coumarinolignans represented by cleomiscosins [7][8]. The structure of **1** was further supported by HMBC and NOESY experiment (*Table 1* and *Fig. 2*). The presence of a phenylpropanoid moiety was confirmed by the long-range correlations between H–C(7′) and C(2′)/(6′), suggesting the presence of the benzodioxin moiety for a coumarinolignan, and also the MeO group with the signal at δ(H) 3.78 was linked to C(6) (δ(C) 145.3). The NOESY correlations between H–C(5′) (*d*, $J = 8$) and *Me*O–C(4′) (*s*) indicating the presence of a 3-hydroxy-4-methoxyphenyl derivative. The large coupling constant, $J = 7.6$ Hz, in **1** could be caused by the inflexible *trans*-configuration between H–C(7′) and H–C(8′). So, the structure of **1** was determined as depicted in *Fig. 1*.

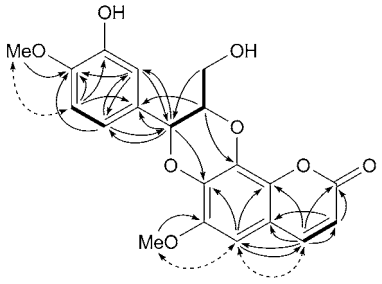


Fig. 2. *Key correlations of compound* **1**. ^{1}H,^{1}H-COSY (—), HMBC (→), and NOESY (dashed arrows)

The NMR spectra of **2** (*Table 2*) were similar to those of **1** except for the result of the NOESY experiment. The correlations between H–C(2′) (*d*, $J = 1.2$) and *Me*O–C(3′) (*s*) suggesting the presence of a 4-hydroxy-3-methoxyphenyl derivative, established compound **2** as cleomiscosin A, which has been isolated from this plant before [6].

The effects of these two compounds on NO inhibition in LPS-induced RAW 264.7 cells were evaluated to investigate the anti-inflammatory activity. Concentrations of nitrite accumulated in the culture medium were estimated by the *Griess* reagent as an index for NO. RAW 264.7 Cells were pretreated with these two compounds at different concentrations. They were found to inhibit LPS-induced NO production with the IC_{50} values of 43.70 μM (compound **1**) and 72.26 μM (compound **2**), compared to the IC_{50} value of the positive control (indomethacin) was 40.13 μM.

Table 1. *^1H- and ^{13}C-NMR Data of* **1** *in (D_6)DMSO* (400 and 125 MHz, resp.; δ in ppm, J in Hz)

Position	δ(H)	δ(C)	HMBC	NOESY
2		160.0		
3	6.34 (*d*, $J = 9.6$)	113.2	C(2), C(10)	H–C(4)
4	7.97 (*d*, $J = 9.6$)	144.8	C(2), C(5), C(9), C(10)	H–C(3), H–C(5)
5	6.92 (*s*)	100.8	C(4), C(7), C(9)	H–C(4), 6-MeO
6		145.3		
7		137.1		
8		131.7		
9		110.7		
10		138.0		
1′		126.7		
2′	7.03 (*d*, $J = 1.2$)	112.3	C(4′), C(6′), C(7′)	H–C(7′), H–C(8′)
3′		146.0		
4′		146.3		
5′	6.80 (*d*, $J = 8.0$)	115.4	C(1′), C(3′)	H–C(6′), 4′-MeO
6′	6.87 (*dd*, $J = 1.2$, 8.0)	120.8	C(2′), C(4′) C(7′)	H–C(5′)
7′	4.99 (*d*, $J = 7.6$)	76.3	C(7), C(1′), C(2′), C(6′)	H–C(2′), H–C(8′)
8′	4.30 – 4.36 (*m*)	77.8	C(8), C(1′)	H–C(2′), H–C(7′)
9′	3.37 – 4.41 (*m*, H_α), 3.63 (br. *s*, $J = 12.4$, H_β)	59.8	C(7′)	H_β–C(9′), H_α–C(9′)
6-MeO	3.78 (*s*)	55.7	C(6)	H–C(5)
4′-MeO	3.79 (*s*)	55.8	C(4′)	H–C(5′)

Table 2. *^1H- and ^{13}C-NMR Data of* **2** *in (D_6)DMSO* (400 and 125 MHz, resp.; δ in ppm, J in Hz)

Position	δ(H)	δ(C)	HMBC	NOESY
2		160.0		
3	6.33 (*d*, $J = 9.6$)	113.2	C(2), C(10)	H–C(4)
4	7.97 (*d*, $J = 9.6$)	144.7	C(2), C(5), C(9), C(10)	H–C(3), H–C(5)
5	6.93 (*s*)	100.6	C(4), C(7), C(9)	H–C(4), 6-MeO
6		145.3		
7		137.1		
8		131.6		
9		110.7		
10		138.3		
1′		126.7		
2′	6.99 (*d*, $J = 1.2$)	112.0	C(4′), C(6′), C(7′)	H–C(7′), H–C(8′), 3′-MeO
3′		147.2		
4′		147.6		
5′	6.82 (*d*, $J = 8.0$)	117.4	C(1′), C(3′)	H–C(6′)
6′	6.88 (*dd*, $J = 1.2$, 8.0)	121.3	C(2′), C(4′) C(7′)	H–C(5′)
7′	5.01 (*d*, $J = 7.6$)	76.2	C(7), C(1′), C(2′), C(6′)	H–C(2′), H–C(8′)
8′	4.30 – 4.36 (*m*)	77.4	C(8), C(1′)	H–C(2′), H–C(7′)
9′	3.35 – 4.39 (*m*, H_α), 3.62 (br. *d*, $J = 12.8$, H_β)	59.9	C(7′)	H_β–C(9′), H_α–C(9′)
6-MeO	3.77 (*s*)	55.7	C(6)	H–C(5)
3′-MeO	3.78 (*s*)	55.8	C(3′)	H–C(2′)

Conclusions. – As a part of our chemical investigations on *Brucea javanica*, a new coumarinolignan cleomiscosin E (**1**) and the known compound cleomiscosin A (**2**) were isolated. Their structures were established on the basis of spectroscopic evidences. The result of bioactivity assay showed that these two compounds exhibited potent anti-inflammatory activities.

Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; 0.2 – 0.5 mm, 0.040 – 0.063 mm, *Merck*, D-Darmstadt) and *Sephadex LH-20* (*Amersham Pharmacia Biotech*, Piscataway, NJ, USA). HPLC: *Waters Alliance 2695* separations module (Empower software) connected to a *Waters 2996* photodiode array (PDA) detector (190 – 800 nm) using a *Sunfire* C_{18} column (150 mm × 4.6 mm, i.d. 5 μm; *Waters*, Milford, MA). Optical rotation: *Rudolph Autopol III* polarimeter (*Rodolph Research Analytical*, New Jersey, USA). UV Spectra (λ_{max} [nm] (log ε)): *UV-260A* spectrophotometer (*Shimadzu*, Tokyo, Japan). IR Spectra ($\tilde{\nu}_{max}$ [cm^{-1}]): *Nicolet 6700* FT-IR instrument (*Thermo Fisher Scientific*, Waltham, MA, USA). NMR Spectra (δ in ppm; *J* values in Hz): *Bruker Avance 400* NMR (*Bruker*, Billerica, MA, USA). MS: *Q-TOF premier mass* spectrometer (*Micromass*, Simonsway, Manchester, UK) coupled with an ESI source in *m*/*z*.

Plant Material. The seeds of *B. javanica* were purchased from a local drug store and were indentified by Dr. *Yanfang Li* (Department of Pharmaceutical Engineering, College of Chemical Engineering, Sichuan University). The voucher specimen (SKLB-200911) was deposited with the laboratory.

Extraction and Isolation. The seeds of *B. javanica* (10 kg) were shattered to powder (*ca.* 20 mesh) and extracted three times with 70% aq. EtOH soln. After evaporation, the extract was suspended in H_2O, and hexane was added to remove less-polar substances. The aq. residue was then extracted with AcOEt to obtain an AcOEt extract (61 g), which was subjected to CC (SiO_2; petroleum ether (PE)/AcOEt from 4 : 1 to 1 : 8) to give nine fractions. *Fr. 5* (4.1 g) was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1) to afford six fractions. *Fr. 5 – 3* (64 mg) was subjected to reversed-phase (RP) prep. HPLC (MeCN and 0.1% aq. HCOOH 25 : 75 (*v*/*v*) to compounds **1** (6 mg) and **2** (14 mg). The duration of the extraction process was *ca.* 1.5 months.

Cleomiscosin E (=*(2*S*,3*S*)-2,3-Dihydro-3-(3-hydroxy-4-methoxyphenyl)-2-(hydroxymethyl)-5-methoxy-9*H*-[1,4]dioxino[2,3-*h*]chromen-9-one*; **1**). Yellow powder. $[\alpha]_D^{21} = -2.3$ ($c = 0.08$ MeOH). UV (MeOH): 204 (4.63), 233 (4.24), 324 (3.90). IR (KBr): 3430, 2939, 1721,1613, 1574, 1444, 1411, 1302, 1217, 1131, 821. ^{1}H- and ^{13}C- NMR: see *Table 1*. HR-EI-MS: 387.1082 ($[M + H]^+$, $C_{20}H_{19}O_8^+$; calc. 387.1080).

Cleomiscosin A (=*(2*S*,3*S*)-2,3-Dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-5-methoxy-9*H*-[1,4]dioxino[2,3-*h*]chromen-9-one*; **2**). Yellow powder. $[\alpha]_D^{23} = -7.3$ ($c = 0.20$ MeOH). UV (MeOH): 208 (4.12), 232 (3.90), 326 (3.45). IR (KBr): 3430, 2932, 1718, 1603, 1576, 1443, 1412, 1304, 1210, 1128, 830. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-EI-MS: 387.1078 ($[M + H]^+$, $C_{20}H_{19}O_8^+$; calc. 387.1080).

Nitrite Assay. The nitrite that accumulated in the culture medium was measured as an indicator of NO production based on the *Griess* reaction. RAW 264.7 Cells were seeded into a 96-well culture plate at a density of 4×10^4 cells per well with 100 μl of culture medium and incubated for 24 h. The cells were then pretreated with compound **1** and **2**, resp., at different concentrations for 2 h before stimulation with LPS (1 μg/ml) for 24 h. The nitrite concentration in the medium was measured according to the *Griess* reaction by adding 50 μl of *Griess* reagent (1% sulfanilamide and 0.1% *N*-(alen-1-)ethylenediamine dihydrochloride in 5% phosphoric acid) to 50 μl of medium for 5 min. The optical density at 540 nm (OD_{540}) was measured with a microplate reader. Concentrations were calculated by comparison with the OD_{540} values of a standard soln. of $NaNO_3$ prepared in culture medium.

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Utilization of Chinese Medicine, Chengdu University of Traditional Chinese Medicine. The authors have declared no conflict of interest.

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