HETEROCYCLES, Vol. 94, No. 11, 2017, pp. 2125 - 2131. © 2017 The Japan Institute of Heterocyclic Chemistry
Received, 30th August, 2017, Accepted, 25th September, 2017, Published online, 28th September, 2017
DOI: 10.3987/COM-17-13805

FLAVONES FROM *CASSIA LESCHENAULTIANA* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITY

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Abstract – Three new (**1**-**3**), together with three known flavones (**4**-**6**), were isolated from the whole plants of *Cassia leschenaultiana*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D NMR techniques. Compounds **1**-**4** were evaluated for their anti-tobacco mosaic virus (anti-TMV) activity. The results showed that compound **3** exhibited high anti-TMV activity with inhibition rate of 48.2%. This inhibition rate is higher than that of positive control (33.8%). Compounds **1**, **2**, and **4** also showed potential anti-TMV activities with inhibition rates of 29.5%, 26.2%, and 27.8%, respectively.

Cassia is a genus of flowering plants in the legume family, Fabaceae, and the subfamily Caesalpinioideae. This genus was a wastebasket taxon for a long time, used to classify plants that did not fit well anywhere else. Over 1000 species have belonged to this genus over the years.[1] More than 10 species of *Cassia* plants are native in China, and more than 20 species were introduced and cultivated in China now.[2] Most of the plant *Cassia* genus has good medicinal value, and this genus had widely been used as traditional

Chinese medicine for treatment of diarrhea, gastritis, ringworm, and fungal skin infections.[2,3] Previous phytochemical studies of genus have shown the presence of anthraquinones,[4,5] steroids,[6,7] chromones,[8-10] terpenes,[11,12] flavonoids,[13-15] alkaloids,[16,17] and the like.

Cassia leschenaultiana DC is an herb plant of the *Cassia* genus, and which is widely distributed in southern China. Its leaves and roots had been used as medicine for dysentery and indigestion, and its seeds have the effect of invigorating stomach, diuresis and eliminating edema.[2] Anthraquinones, steroids, flavonoids, and terpenes in this plant had also been reported in the previous literatures.[18,19] In our continuing efforts to identify bioactive natural products from *Cassia* genus, we now investigated the chemical constituents of the whole plant of *C. leschenaultiana*. This leads to the isolation of three new (**1**-**3**), and three known flavones (**4**-**6**). The structures of **1**-**6** were elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. Compounds **1**-**4** were also evaluated for their anti-tobacco mosaic virus (anti-TMV) activities. This article deals with the isolation, structural elucidation and biological activities of these compounds.

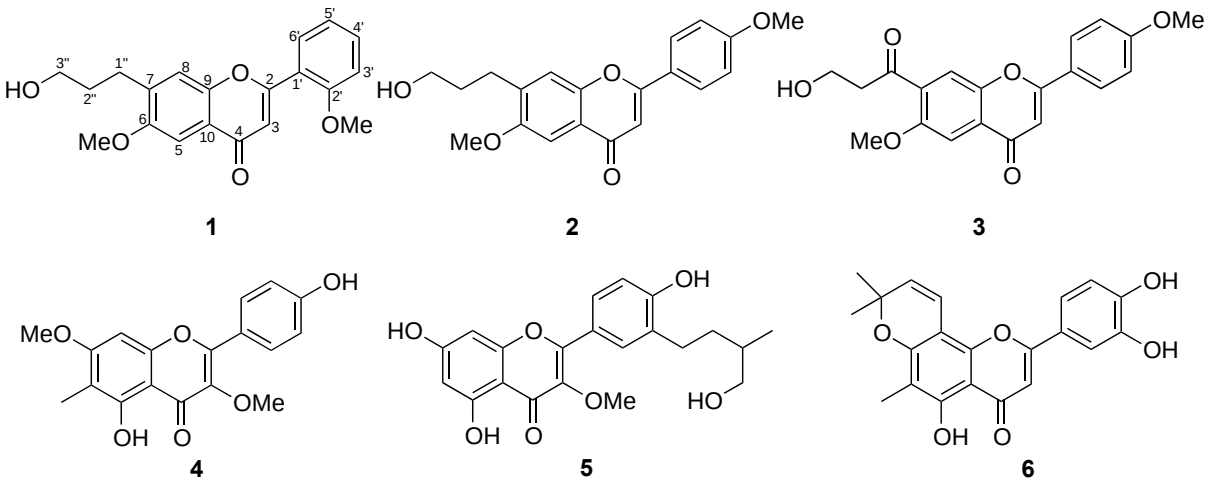

Figure 1. The structures of flavones form the whole plants of *C. leschenaultiana*

The whole plants of *C. leschenaultiana* were extracted with 95% methanol (MeOH), followed by repeated column chromatography on silica gel, Sephadex LH-20 and RP-18 silica gel. Final purification by semi-preparative RP-HPLC afforded six flavones (**1**-**6**). The structures of **1**-**6** are shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1**-**3** are given in Table 1. The known compounds were identified as 5,4′-dihydroxy-3,7-dimethoxy-6-methylflavone (**4**),[20] dodoviscin I (**5**),[21] and desmodol (**6**).[22]

Compound **1** was obtained as an orange-yellow gum. It has the molecular formula $C_{20}H_{20}O_5$ from HRESIMS (*m/z*: 363.1215 $[M+Na]^+$, calcd 363.1208), with 11 degrees of unsaturation. Its IR spectral data showed the presence of hydroxy group (3428 cm^{-1}), carbonyl group (1665 cm^{-1}), and aromatic ring

(1618, 1552, and 1463 cm^{-1}). The UV absorptions at 358, 256, and 210 nm also showed a substituted aromatic ring. The ^{1}H and ^{13}C NMR spectrum of **1** (Table 1, Figure S 1-8) along with analysis of the DEPT spectra displayed 20 carbon signals and 20 proton signals, respectively, corresponding to a 1,2,4,5-tetrasubstituted aromatic ring (C-5 ~ C-10; H-5 and H-8), a 1,2-disubstituted aromatic ring (C-1′ ~ C-6′; H-3′ ~ H-6′), an *α,β*-unsaturated ketone (C-2 ~ C-4; H-3), a hydroxypropyl group (C-1″ ~ C-3″; H_2-1″ ~ H_2-3″),[23] two methoxy groups (δ_C 56.2 q, 55.9 q, δ_H 3.81 s, 3.79 s), and a hydroxy group (δ_H 4.91 s). The typical NMR signal of two aromatic ring and *α,β*-unsaturated ketone indicated that **1** should be a flavone.[24] This was also supported by the HMBC correlations (Figure 2) from H-3 to C-2, C-4, C-10, C-1′, from H-5 to C-4, C-9, C-10, from H-8 to C-9, C-10, and from H-6′ to C-2. Since the nucleus of compound was determined, the additional signals (one hydroxypropyl and two methoxy groups) were accounted for the remaining substituents. The HMBC of correlations of two methoxy protons (δ_H 3.81, 3.79) with C-6 (δ_C 153.3) and C-2′ (δ_C 156.5) suggested the attachment position of the two methoxy groups at C-6 and C-2′, respectively. The hydroxypropyl group located at C-7 was supported by the HMBC of correlations from H_2-1″ (δ_H 2.72) to C-6 (δ_C 153.3), C-7 (δ_C 130.3), C-8 (δ_C 117.0), from H_2-2″ (δ_H 1.89) to C-7 (δ_C 130.3), and from H-8 (δ_H 6.80) to C-1″ (δ_C 29.0). Furthermore, the typical protons signals (H-5, H-8, H-3′~H-6′) also supported a 6,7-disubstituted for ring A and a 2′-monosubstituted for ring B on flavone nucleus. The structure of 7-(3-hydroxypropyl)- 6,2'-dimethoxyflavone (**1**) was therefore established as shown in Figure 1.

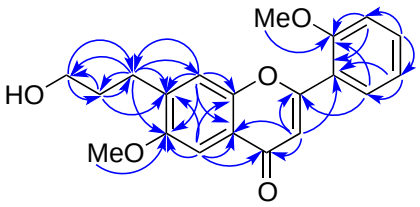

Figure 2. Key HMBC correlations (↷) of **1**

The ^{1}H and ^{13}C NMR spectra of 7-(3-hydroxypropyl)-6,4'-dimethoxyflavone (**2**) were similar to those of **1**. The marked differences between them were due to the substituent position change on B ring. The NMR data for B ring (C-1′, δ_C 123.5 s, C-2′,6′, δ_C 131.3 d, C-3′,5′, δ_C 115.5 d, and C-4′, δ_C 161.1 s; H_2-2′,6′, δ_H 7.76 (d) 8.8 Hz, H_2-3′,5′, δ_H 6.85 (d) 8.8 Hz) suggested a methoxy group should be located at C-4′. This was also supported by the HMBC correlation of methoxy protons (δ_C 3.78) with C-4′ (δ_C 161.1). In addition, the other methoxy located at C-6, and the 3-hydroxypropyl located at C-7 also determined by the further analysis of its HMBC correlations. Thus, the structure of **2** was determined as shown.

Compound **3** was isolated as an orange-yellow gum and it gave an $[M+Na]^+$ peak at *m/z* 377.1008, consistent with a molecular formula of $C_{20}H_{18}NaO_6$, with 12 degrees of unsaturation. Its ^{1}H and ^{13}C NMR spectroscopic data were similar to those of **2**, which suggested that compound **3** was also structurally related to **2**. The marked differences between them were due to the inexistence of a 3-hydroxypropyl group, and appearance of a 3-hydroxypropanoyl group[26] (C-1″ and C-3″; H_2-2″ and H_2-3″) in compound

3. These changes indicated that a 3-hydroxypropyl group in **2** was replaced by a 3-hydroxypropanoyl group in compound **3**. This deduction was also supported by the HMBC correlations from H_2-2″ (δ_H 2.93) to C-7 (δ_C 127.2), and from H-8 (δ_H 7.44) to C-1″ (δ_C 199.9). Morever two methoxy groups located at C-6 and C-4′ was supported by the HMBC correlations of two methoxy proton signals (δ_H 3.81, 3.79) with C-6 and C-4′, respectively. Based on the above findings, the structure of **3** was formulated as 7-(3-hydroxypropanoyl)-6,4'-dimethoxyflavone.

Table 1. ^{1}H and ^{13}C NMR data for compounds **1-3** ($CDCl_3$, 125 and 500 MHz)

No.	Compound **1**		Compound **2**		Compound **3**	
	δ_C (mult.)	δ_H (mult, *J*, Hz)	δ_C (mult.)	δ_H (mult, *J*, Hz)	δ_C (mult.)	δ_H (mult, *J*, Hz)
2	163.1 s		163.2 s		163.5 s	
3	106.4 d	6.63 s	106.6 d	6.64 s	106.3 d	6.62 s
4	177.1 s		177.4 s		177.6 s	
5	113.7 d	7.19 s	113.9 d	7.16 s	113.0 d	7.20 s
6	153.3 s		153.5 s		155.9 s	
7	130.3 s		130.5 s		127.2 s	
8	117.0 d	6.80 s	116.6 d	6.81 s	117.9 d	7.44 s
9	149.1 s		149.3 s		148.6 s	
10	122.7 s		122.6 s		128.8 s	
1′	118.9 s		123.5 s		123.6 s	
2′	156.5 s		131.3 d	7.76 (d) 8.8	131.0 d	7.74 (d) 8.8
3′	115.9 d	6.93 (d) 7.6	115.5 d	6.85 (d) 8.8	115.6 d	6.85 (d) 8.8
4′	131.0 d	7.36 (t) 7.6	161.1 s		160.8 s	
5′	121.2 d	6.88 (t) 7.6	115.5 d	6.85 (d) 8.8	115.6 d	6.85 (d) 8.8
6′	128.2 d	7.82 (d) 7.6	131.3 d	7.76 (d) 8.8	131.0 d	7.74 (d) 8.8
1″	29.0 t	2.72 (t) 7.8	30.2 t	2.72 (t) 7.8	199.9 s	
2″	37.2 t	1.89 m	37.6 t	1.92 m	43.3 t	2.93 (t) 6.4
3″	63.4 t	3.56 (t) 6.6	63.6 t	3.57 (t) 6.6	58.4 t	4.16 (t) 6.4
-OMe-6	56.2 q	3.81 s	56.3 q	3.80 s	56.2 q	3.81 s
-OMe-2′	55.9 q	3.79 s				
OMe-4′			56.0 q	3.78 s	55.9 q	3.79 s
-OH-3″		4.91 s		4.93 s		4.93 s

Since certain of the flavones from plants of *Cassia* genus exhibit potential anti-TMV activity,[13,27,28] compounds **1-4** were tested for their anti-TMV activity. The inhibitory activity of compounds **1-4** against TMV replication were tested using the half-leaf method at the concentration of 20 μM.[29,30] Ningnanmycin (with inhibition rate of 34.8%), a commercial product for plant disease in China, was used as a positive control. The results showed that compound **3** exhibited high anti-TMV activity with inhibition rate of 48.2%. This inhibition rate is higher than that of positive control (33.8%). Compounds **1**, **2**, and **4** also showed potential anti-TMV activities with inhibition rates of 29.5%, 26.2%, and 27.8%, respectively (Figure S9).

Since the inhibition rate of compound **3** is higher than that of positive control, its IC_{50} values was tested with ningnanmycin as the positive control. The results revealed that compound **3** exhibited the good activity, with an IC_{50} value of 21.2 μM; the efficiency was higher than that of ningnamycin (35.8 μM). In addition, the protective effects of compound **3** on TMV were also evaluated by pretreating the tobacco plant with 20 μM solutions of compounds or a solution of DMSO for 6 h before inoculation with TMV. The results showed that compound **3** showed protective effects to the host plants with the inhibition rate 52.2%. This results indicated that pretreatment with compound **3** could increase the resistance of the host plant to TMV infection (Figure S10).

General Experimental Procedures. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectra. 1D- and 2D- NMR spectroscopic data were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the TMS signal. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semi-preparative HPLC was performed on an Agilent 1200 preparative liquid chromatograph with Agilent Zorbax PrepHT GF (21.2 mm × 25 cm) or Agela Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Inc, USA), or MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC visualized by spraying with 5% H_2SO_4 in ethanol and heating.

Plant Material. The whole plants of *Cassia leschenaultiana* DC were collected from Yuanjiang County of Yuxi Prefecture, Yunnan province in September 2016. The species was identified by Prof. Chen Y. J. A voucher specimen (YNNI 16-9-103) was deposited in Key Laboratory of Chemistry in Ethnic Medicinal Resources, Yunnan Minzu University.

Extraction and Isolation. The dryed samples (4.8 kg) were crushed to 30 mesh, and the powders were extracted with 95% aqueous MeOH (4 × 8 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure, and then extracted with EtOAc. The EtOAc-soluble materials (98.6 g) were applied to silica gel (150-200 mesh) column eluted with $CHCl_3$-MeOH gradient elution (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to afford six fractions (A-F). Further separation of fraction B (9:1, 13.8 g) by silica gel column chromatography, eluted with $CHCl_3$-acetone (1:0-1:2), yielded subfractions B1–B7. Subfraction B3 (8:2, 3.85 g) was loaded on to another silica gel column using petroleum ether-EtOAc elution, and then separated semi-preparative HPLC (65% MeOH, flow rate 20 mL/min) to afford **1** (12.2 mg), **2** (13.9 mg), **3** (10.8 mg), **5** (15.6 mg), and **6** (18.9 mg). Subfraction B4 (7:3, 4.27 g) was separated on a silica gel column eluted by petroleum ether-EtOAc, followed by semi-preparative HPLC (58% MeOH, flow rate 20 mL/min) to give **4** (36.2 mg).

7-(3-Hydroxypropyl)-6,2'-dimethoxyflavone (1): $C_{20}H_{20}O_5$, orange-yellow gum; UV (MeOH) λ_{max} (log ε) 358 (3.73), 256 (3.95), 210 (4.33) nm; IR (KBr): ν_{max} 3428, 2857, 1665, 1618, 1552, 1463, 1150, 1258, 1150, 1064 cm^{-1}; ^1H and ^{13}C NMR data (500 and 125 MHz), see Table 1; ESIMS *m/z* 363; HRESIMS *m/z* 363.1215 $[M+Na]^+$ (calcd for $C_{20}H_{20}NaO_5$ 363.1208).

7-(3-Hydroxypropyl)-6,4'-dimethoxyflavone (2): $C_{20}H_{20}O_5$, orange-yellow gum; UV (MeOH) λ_{max} (log ε) 362 (3.74), 263 (3.78), 210 (4.22) nm; IR (KBr): ν_{max} 3416, 2863, 1665, 1612, 1562, 1449, 1162, 1257, 1136, 1050 cm^{-1}; ^1H and ^{13}C NMR data (500 and 125 MHz), see Table 1; ESIMS *m/z* 363; HRESIMS *m/z* 363.1202 $[M+Na]^+$ (calcd for $C_{20}H_{20}NaO_5$, 363.1208).

7-(3-Hydroxypropanoyl)-6,4'-dimethoxyflavone (3): $C_{20}H_{18}O_6$, orange-yellow gum; UV (MeOH) λ_{max} (log ε) 368 (3.81), 269 (3.92), 210 (4.40) nm; IR (KBr) ν_{max} 3430, 1686, 1663, 1610, 1571, 1452, 1265, 1157, 1069 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 377 $[M+Na]^+$, HRESIMS *m/z* 377.1008 $[M+Na]^+$ (calcd for $C_{20}H_{18}NaO_6$, 377.1001).

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 21562044 and 81660717), the Research Foundation of China Tobacco Company (No. 110201502006), the Research Foundation of China Tobacco Yunnan Industrial Co., Ltd (No. 2015539200340277 and 2016JC03), and the Natural Science Foundation of Yunnan Province (No. 2014FD033).

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