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CHROMONE DERIVATIVES FROM THE TWIGS OF *CASSIA AGNES* AND THEIR ANTI-ROTAVIRUS ACTIVITY

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Abstract – Three new (**1 - 3**), together with four known chromone derivatives (**4 - 7**) were isolated from the twigs of *Cassia agnes*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D-NMR techniques. Compounds **1 - 7** were evaluated for their anti-rotavirus activity. The results revealed that compounds **1 - 7** exhibited potent anti-rotavirus activity with TI valves in the range of 9.4 - 14.5, respectively.

Cassia is a genus of flowering plants in the legume family, Fabaceae, and the subfamily Caesalpinioideae.[1] This genus represents one of the largest and most diverse group of flowering plants, including herbs to trees. The plants of the genus *Cassia* are widely distributed in most tropical and subtropical countries. More than 10 species of Cassia plants are native in China, and more than 20 species were introduced and cultivated in China now.[2] Because they have the biological and medical activities such as hepatoprotective, antibacterial, antioxidant, antitumor, antidiabetic, and antiparasitic, some of Cassia plants had widely been used as traditional Chinese medicine.[3-6] In addition, the previous phytochemical studies revealed that the anthraquinones,[7,8] steroids,[9,10] chromones,[11-13] terpenes,[14,15] flavonoids,[16,17] alkaloids,[18,19] and the like, had been isolated from the plants of this genus.

Cassia agnes (de Wit) Brenan is an perennial sub-shrub herb plant of the *Cassia* genus, and which is widely distributed in southern China.[1] This plant has the effect of clearing heat, detoxifying, and defecating, and had been used as medicine for measles, varicella, cold, stomachache, and constipation.[20]

Anthraquinones, flavonoids, steroids, and terpenes in this plant had also been reported in the previous literatures.[20-22] Since plants have played a major role in the introduction of new therapeutic agents, to the best of our knowledge, we now investigated the chemical constituents of the whole plant of *C. agnes*. This led to the isolation of three new (**1** - **3**) together with four known (**4** - **7**) chromone derivatives. The structures of **1** - **7** had been elucidated by spectroscopic methods including extensive 1D- and 2D-NMR techniques, and their anti-rotavirus activity had also been evaluated.

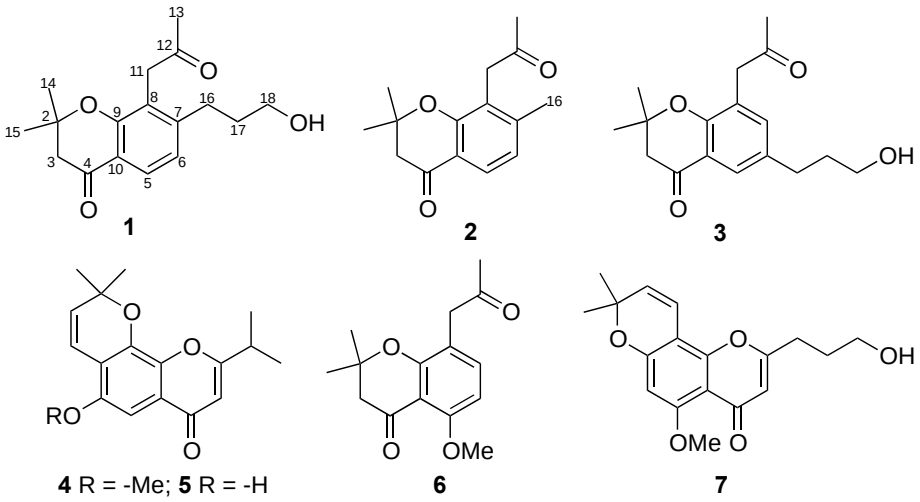

Figure 1. The chromone derivatives from *C. agnes*

The air-dried twigs of *C. agnes* were extracted with 95% methanol (MeOH), followed by repeated column chromatography on silica gel, Sephadex LH-20 and RP-18. Final purification by semi-preparative RP-HPLC afforded three new chromone derivatives, 7-(3-hydroxypropyl)-2,2-dimethyl-8-(2-oxopropyl)-chroman-4-one (**1**), 2,2,7-trimethyl-8-(2-oxopropyl)chroman-4-one (**2**), 6-(3-hydroxypropyl)-2,2-dimethyl-8-(2-oxopropyl)chroman-4-one (**3**), together with four known chromone derivatives (**4** - **7**). The structures of compounds **1** - **7** were shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1** - **3** were given in Table 1. The known compounds, compared with the literature, were identified as siamchromone S (**4**),[12] siamchromone T (**5**),[12] 5-methoxy-2,2-dimethyl-7-(2-oxopropyl)-2,3-dihydrochromen-4-one (**6**),[23] and siamchromone G (**7**).[24]

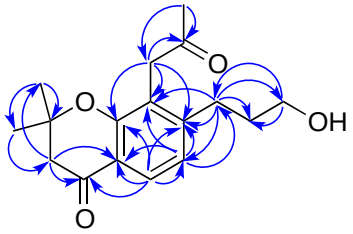

Figure 2. Key HMBC (↷) correlations of **1**

Compound **1** was obtained as yellow gum after purified by preparative HPLC. This compound gave a parent ion by HR-MS at *m/z* 313.1422 $[M+Na]^{+}$ (calculated for 313.1416, $C_{17}H_{22}NaO_4$), corresponding to a molecular formula $C_{17}H_{22}O_4$, and requiring seven degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **1** along with analysis of the DEPT spectra (Table 1)

displayed 17 carbon signals and 22 proton signals, respectively. These signals corresponding to an 1,2,3,4-tetrasubstituted benzene ring (C-5 ~ C-10, H-5, and H-6, including an oxidized carbon), one 2-oxopropyl group (-CH_2CO-Me, C-11~C-13, H_2-11, H_3-13),[23] one 3-hydroxypropyl group (C-16~C-18, H_2-16~H_2-18),[24] one *gem*-dimethyl carbon (C-14,15 and H_6-14,15), one carbonyl carbon (C-4), one methylene carbon (C-3 and H_2-3), and one quaternary carbon (C-2). Strong absorption bands accounting for hydroxy (3924 cm^{-1}), carbonyl group (1712 and 1672 cm^{-1}), and aromatic groups (1618, 1565, 1442 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 354, 258, and 215 nm, which confirmed the existence of the aromatic functions. In addition to benzene ring, there is still existed on ring in the molecule to support the seven degrees of unsaturation, and the typical signals for one oxidized carbon on benzene ring (C-9), carbonyl (C-4), *gem*-dimethyl (C-14,15), methylene (C-3), and quaternary carbon (C-2) should be formed a *gem*-dimethylchromone nucleus.[23] This deduction was also supported by the HMBC correlations (Figure 2) from H-5 to C-4, C-9, C-10, and from H-3 to C-10.

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

No.	Compound **1**		Compound **2**		Compound **3**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
2	77.9 s		78.3 s		78.2 s	
3	48.8 t	2.66 s	50.1 t	2.66 s	49.0 t	2.66 s
4	192.6 s		191.9 s		192.3 s	
5	126.9 d	7.56 (d) 8.2	126.6 d	7.50 (d) 8.2	125.1 d	7.60 (d) 1.8
6	119.6 d	6.83 (d) 8.2	118.6 d	6.79 (d) 8.2	130.1 s	
7	136.3 s		138.7 s		134.8 d	7.09 (d) 1.8
8	128.4 s		129.5 s		126.3 s	
9	155.9 s		156.9 s		154.4 s	
10	116.8 s		116.5 s		119.2 s	
11	44.3 t	3.91 s	44.6 t	3.93 s	48.3 t	3.90 s
12	205.9 s		205.7 s		204.9 s	
13	30.0 q	2.24 s	29.7 q	2.10 s	29.7 q	2.22 s
14,15	26.4 q	1.60 s	26.1 q	1.60 s	25.8 q	1.61 s
16	32.6 t	2.75 (t) 7.8	18.6 q	2.36 s	34.7 t	2.73 (t) 7.8
17	38.5 t	1.85 m			38.0 t	1.88 m
18	63.6 t	3.50 (t) 6.6			63.9 t	3.54 (t) 6.6

Since the nucleus of compound was determined, the additional carbons (3-hydroxypropyl and 2-oxopropyl group) were accounted for the remaining substituents. The HMBC correlations between H_2-11 and C-7, C-8 and C-9 indicated that the 2-oxopropyl group should be located at C-8 on the chromone ring. The 3-hydroxypropyl group located at C-7 was supported by the HMBC correlations from

H_2-16 to C-6, C-7, C-8, and from H_2-17 to C-7. Thus, the structure of **1** was established as shown, and given the systematic name of 7-(3-hydroxypropyl)-2,2-dimethyl-8-(2-oxopropyl)chroman-4-one.

2,2,7-Trimethyl-8-(2-oxopropyl)chroman-4-one (**2**) was obtained as yellow oil with molecular formula $C_{15}H_{18}O_3$ as determined by positive HRESI-MS (*m/z* 269.1160 $[M+Na]^+$). Its ^1H and ^{13}C NMR spectra were similar to those of **1**. The marked differences between them were due to the inexistence of a 3-hydroxypropyl group signals, and appearance of a methyl group signals (C-16 and H_3-16). The 2D-HMBC data of three- and two-bond correlations from H_3-16 to C-6, C-7, C-8, and from H-6 to C-16 revealed that the methyl group located at C-7. The 2-oxopropyl group located at C-8 was also supported by the HMBC correlations between H-11 and C-7, C-8 and C-9. On the basis of the above evidence, compound **2** was assigned as 2,2,7-trimethyl-8-(2-oxopropyl)chroman-4-one.

Compound **3** was also obtained as yellow gum, and showed quasi molecular ion at *m/z* 313.1412 $[M+Na]^+$ in the HRESIMS (calcd 313.1416, for $C_{17}H_{22}NaO_4$), corresponding to the molecular formula of $C_{17}H_{22}O_4$. The ^1H and ^{13}C NMR spectra of **3** were also similar to those of **1**. The obvious chemical shift differences resulted from the proton signals on benzene ring. The typical signals (7.56 (d) 8.2 Hz and 6.83 (d) 8.2 Hz) were replaced by (7.50 (d) 1.8 Hz and 6.79 (d) 1.8 Hz) in **1**, and this indicted that the substituent positions had been varied on the chromone nucleus, and **3** should be a 6,8-disubstituted chromone. In addition, the 2-oxopropyl group attached to C-8 and the 3-hydroxypropyl group attached to C-6 was supported by the HMBC correlations from H_2-11 to C-7, C-8, C-9, from H_2-16 to C-5, C-6, C-7, and from H_2-17 to C-6 respectively. Thus, the structure of 6-(3-hydroxypropyl)-2,2-dimethyl-8-(2-oxopropyl)chroman-4-one (**3**) was established as shown.

Table 2. Anti-rotavirus activity of compounds **1-7**

No.	CC_{50} (μg/mL)	EC_{50} (μg/mL)	TI (CC_{50}/EC_{50})
1	186.7	13.8	13.5
2	212.4	14.6	14.5
3	203.5	12.2	16.7
4	147.5	11.9	12.4
5	182.2	15.4	11.8
6	157.9	16.8	9.4
7	164.3	14.0	11.7
Ribavirin	280.5	14.2	19.8

CC_{50}: mean (50%) value of cytotoxic concentration; EC_{50}: mean (50%) value of effective concentration; TI: therapeutic index, CC_{50}/EC_{50}.

Since certain chromones from *Cassia* genus exhibit potential antiviral activity,[12,13,23,24] compounds **1 - 7** were tested for their anti-rotavirus activity. Their ability to prevent the cytopathic effects of rotavirus in MA104 cells was tested according to our previous literatures,[25,26] and their effects were measured in parallel with the determination of antiviral activity using ribavirin as positive control. The results (Table 2) revealed that compounds **1-7** exhibited potent anti-rotavirus activity with therapeutic index (TI) valve in the range of 9.4-14.5.

EXPERIMENTAL

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 1260 preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or 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 twigs of *Cassia agnes* (de Wit) Brenan were collected from Yuanjiang Prefecture, Yunnan province in September 2018. The species was identified by Prof. Y. J. Chen. A voucher specimen (YNNI 18-9-22) was deposited in Key Laboratory of Chemistry in Ethnic Medicinal Resources, Yunnan Minzu University.

Extraction and Isolation. The air-dried samples (5.4 kg) were crushed to 30~50 mesh, and the powders were extracted with 95% MeOH (8 L × 4) at room temperature and filtered. The filtrate was evaporated under reduced pressure, and the crude extract (682 g) was applied to a silica gel (150-200 mesh) column eluted with chloroform-methanol ($CHCl_3$-MeOH) gradients (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, 48.9 g) by silica gel column chromatography, eluted with $CHCl_3$-acetone (1:0-1:2), yielded subfractions B1 – B7. Subfraction B3 (8:2, 11.6 g) was separated on the other silica gel column eluted by petroleum ether-EtOAc, followed by semi-preparative HPLC (55% MeOH-H_2O, flow rate 20 mL/min) to give **1** (14.4 mg), **3** (16.2 mg), **4** (18.2 mg), and **7** (22.3 mg). Subfraction B4 (7:3, 12.4 g) was separated on another silica gel column eluted by petroleum ether-EtOAc, followed by semi-preparative HPLC (48% MeOH-H_2O, flow rate 20 mL/min) to give **2** (18.4 mg), **5** (22.1 mg), and **7** (16.6 mg).

Anti-rotavirus assay. The human rotavirus Wa group was used to infect the cell culture MA104 *in vitro*, the 50% cytotoxicity concentration (CC_{50}) and half maximal effective concentration (EC_{50}) were evaluated, and the ribavirin was used as positive control.[25,26] MA-104 cells (1×10^5 cells *per* well) were grown in 96-well plates for 48 h. The media were removed and replaced by new media containing serial dilutions of compounds under test. After incubation for 72 h, the media were discarded, and 5 μL of MTT solution was added to each well. Plates were then incubated at 37 °C for 4 h. The solution was removed, and 100 μL of 0.04 mol/L HCl-isopropanol were added to each well to dissolve formazan crystals. Using a microplate reader, the absorbance of each well was measured at 540 nm. After subtracting the background absorbance at 655 nm, the 50% CC_{50} of each compound was estimated by regression analysis.

7-(3-Hydroxypropyl)-2,2-dimethyl-8-(2-oxopropyl)chroman-4-one (1). $C_{17}H_{22}O_4$, obtained as pale yellow gum; UV (MeOH) max (log ε) 215 (4.08), 258 (3.67), 354 (3.31) nm; IR (KBr) ν_{max} 3924, 3051, 2962, 2840, 1712, 1672, 1618, 1565, 1442, 1356, 1162, 906 cm^{-1}; ^1H NMR and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1; positive ESIMS *m/z* 313 $[M+Na]^+$; positive HRESIMS *m/z* 313.1422 $[M+Na]^+$ (calcd 313.1416, for $C_{17}H_{22}NaO_4$).

2,2,7-Trimethyl-8-(2-oxopropyl)chroman-4-one (2). $C_{15}H_{18}O_3$, obtained as pale yellow oil; UV (MeOH) max (log ε) 212 (4.15), 256 (3.76), 350 (3.28) nm; IR (KBr) ν_{max} 3036, 2945, 2838, 1716, 1678, 1622, 1553, 1430, 1362, 1169, 873 cm^{-1}; ^1H NMR and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1; positive ESIMS *m/z* 269 $[M+Na]^+$; positive HRESIMS *m/z* 269.1160 $[M+Na]^+$ (calcd 269.1154, for $C_{15}H_{18}NaO_3$).

6-(3-Hydroxypropyl)-2,2-dimethyl-8-(2-oxopropyl)chroman-4-one (3). $C_{17}H_{22}O_4$, obtained as pale yellow gum; UV (MeOH) max (log ε) 215 (4.12), 260 (3.74), 352 (3.38) nm; IR (KBr) ν_{max} 3952, 3056, 2950, 2863, 1714, 1670, 1620, 1555, 1463, 1352, 1161, 885 cm^{-1}; ^1H NMR and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1; positive ESIMS *m/z* 313 $[M+Na]^+$; positive HRESIMS *m/z* 313.1412 $[M+Na]^+$ (calcd 313.1416, for $C_{17}H_{22}NaO_4$).

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