Asian Journal of Chemistry; Vol. 24, No. 11 (2012), 4892-4894

ASIAN JOURNAL OF CHEMISTRY

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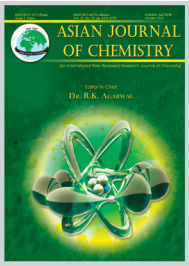

Flavonoids from the Fruits of *Camellia oleifera*

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(*Received*: 25 June 2011; *Accepted*: 28 May 2012) AJC-11521

A new flavonoid, 8-formyl-3',4',5'-trihydroxy-6,7-dimethoxyflavonoid (**1**), together with eight flavonoids (**2-8**) were isolated from the fruits of *Camellia oleifera*. The structures of compounds **1-9** were elucidated on the basis of extensive NMR and MS means. The anti-tobacco mosaic virus and antioxidant activity of **1** were evaluated. It showed anti-tobacco mosaic virus activity with inhibition rates of 85.7 % and antioxidant activity with an IC_{50} value of 2.78 mg/mL.

Key Words: Flavonoids, *Camellia oleifera*, Anti-tobacco mosaic virus activity, Antioxidant activity.

INTRODUCTION

Camellia oleifera Abel., a theaceous evergreen tree, is widely distributed and long time cultivated in south China. The seeds are important oil material for producing a kind of cooking oil (tea oil), whose beneficial unsaturated fatty acids are comparable to those of olive oil[1]. To obtain one ton of tea oil, four times of residue composing of the remaining grounded fruit and shell will be produced, which is called 'seed cake of *C. oleifera*. As a big amount of by-product, the seed cake of *C. oleifera* is normally used as detergent, animal feeds or organic fertilizer, due to the containing of rich polyphenols, saponins, protein, polysaccharide, *etc*.[2-5]. With the aim of continuing efforts to identify bioactive natural products from the plants, a chemical investigation on the fruits of *C. oleifera* indigenous to the Dehong Prefecture of Yunnan Province, P.R. China, was carried out and a new flavonoid (**1**), together with nine known flavonoids (**2-9**) were isolated from the fruits of this plant. In addition, the anti-tobacco mosaic virus (anti-TMV) and antioxidant activity of compound **1** were evaluated. Described in this paper are their structure elucidation and biological activities.

EXPERIMENTAL

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 and ^2D NMR spectra were recorded on Bruker DRX-500 instruments with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10-40 mm, Qingdao Marine Chemical Inc., China). On second separate used Agilent 1100 HPLC equipped with ZORBAX-C_{18} (9.4 × 250 nm, 5.0 μm) column and DAD detector.

The fruits of *Camellia oleifera* Abel. was collected in Dehong Prefecture, Yunnan Province, P.R. China, in October 2010 and was identified by Prof. N. Yuan. A voucher specimen (No. YNNi 09-2-02) was deposited in our laboratory.

Extraction and isolation: The air-dried and powdered fruits of *C. oleifera* (1.5 kg) were extracted with 70 % aqueous ethanol (3.0 L × 3, 24 h each) at room temperature and the extract was concentrated under vacuum condition. The dried extract (102 g) was applied to Si gel (200-300 mesh) column chromatography eluting with a $CHCl_3$- Me_2CO gradient system (9:1, 8:2, 7:3, 6:4, 5:5, 2:1) to give six fractions A-F. The separation of fraction C ($CHCl_3$-Me_2CO 7:3, 18.6 g) by Si gel column chromatography eluted with $CHCl_3$-Me_2OH (9:1-1:2) yielded mixtures C1-V5. Fraction C2 (1.87 g) was subjected to preparative HPLC (35 % MeOH-H_2O or 22 % CH_3CN-H_2O, flow rate 12 mL/min) to give 1 (15.6 mg), **2** (54.5 mg) and **3** (32.5 mg). The separation of fraction D ($CHCl_3$- Me_2CO 1:1, 28.4 g) by Si gel column chromatography eluted with $CHCl_3$-Me_2OH (9:1-1:2) yielded mixtures D1-D5. Fraction D3 (3.76 g) was subjected to preparative HPLC (15 % MeOH-H_2O or 10 % CH_3CN-H_2O, flow rate 12 mL/min) to give **4** (36.4 mg) **7** (11.6 mg) and **8** (60.8 mg). Fraction D4 (5.26 g) was subjected to preparative HPLC (10 % MeOH-H_2O or 7.5 % CH_3CN-H_2O, flow rate 12 mL/min) to give **5** (36.4 mg), **6** (28.4 mg) and **9** (50.2 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[6].

Anti-TMV assays: The anti-tobacco mosaic virus actives were tested using the half-leaf method[7]. The inhibitory activities of the compound **1** against tobacco mosaic virus replication were tested using two approaches. First, the half-leaf method was used to test the antiviral activity in the local lesion host *N. glutinosa in vivo*. Then, the leaf-disk method was used to evaluate the antiviral activity of the compound in the systemic infection host *N. tabacum* cv. K326. Ningnanmycin, a commercial product for plant disease in China, was used as a positive control.

Spectral data

8-Formyl-3',4',5'-trihydroxy-6,7-dimethoxyflavonoid (1): $C_{18}H_{14}O_8$, orange-yellow gum; UV (CH_3OH), λ_{max} (log ε) 210 (4.95), 258 (4.22), 292 (3.80), 370 (4.15) nm; IR (KBr, ν_{max}, cm^{-1}) 3464, 1685, 1650, 1624, 1522, 1480, 1465, 1127, 1095, 952, 870; ^{13}C NMR and ^1H NMR data (C_5D_5N, 500 MHz) (Table-1); positive ESIMS *m/z* 381 $[M+Ma]^+$; HRESIMS *m/z* 381.0586 $[M+Na]^+$ (calcd. for $C_{18}H_{14}O_8Na$, 381.0580).

RESULTS AND DISCUSSION

A 70 % aq. ethanol extract prepared from the fruits of *C. oleifera* was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford 8-formyl-3',4',5'-trihydroxy-6,7-dimethoxyflavonoid (**1**), kaempferol (**2**)[8], quercetin (**3**)[8], quercetin 3-*O*-*β*-glucopyranoside (**4**)[8], leucoside (**5**)[9], kaempferol-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D- glucopyranoside (**6**)[10], isovitexin (**7**)[11], luteolin-6-C-*β*-D-glucopyranoside (**8**)[12], isosaponarin (**9**)[12]. The structure of the **1-9** was shown in Fig. 1 and the ^1H and ^{13}C NMR spectroscopic data of **1** were listed in Table-1.

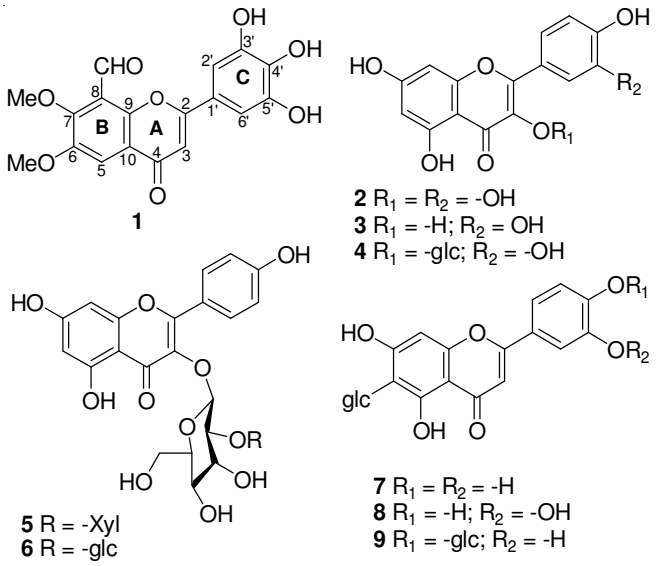

Fig. 1. Flavonoids from the fruits of *Camellia oleifera*

Compound **1** was obtained as an orange-yellow gum. It has the molecular formula $C_{18}H_{14}O_8$ from HRESIMS (*m/z*: 381.0580 $[M+Na]^+$, calcd. 381.0586). The ^1H and ^{13}C NMR spectrum of **1** (Table-1) along with analysis of the DEPT spectra displayed 18 carbon signals and 14 proton signals, respectively, corresponding to a flavonoid nucleus[14] (δ_C 164.8, 106.2, 178.5, 122.8, 147.2, 159.1, 115.6, 152.1, 121.3, 123.1, 106.8, 147.5, 138.2, 147.5, 106.8), one aldehyde group (δ_C 192.6; δ_H 10.22), two methoxy groups (δ_C 55.8, 61.8; δ_H 3.80, 3.82) and two low-field hydroxyl protons (δ_H 10.92 2H, 11.26 1H). The typical NMR signals at (δ_H 7.06 s, 2H), (δ_C 123.1 s, 106.8 d, 147.5 s, 138.2 s) observed in the ^1H NMR spectrum revealed that the aromatic ring C of **1** is a symmetrical 1,3,4,5-tetra-substituted phenolic moiety[14] and the HMBC of correlations (Fig. 2) of the hydroxyl proton signals, δ_H 10.92 with C-2' (δ_C 106.8), C-3' (δ_C 147.5), C-4' (δ_C 138.2) and δ_H 11.26 with C-3' (δ_C 147.5), C-4' (δ_C 138.2), C-5' (δ_C 147.5) suggested the attachment position of the three hydroxy groups at C-3', C-4' and C-5'. Since the substituents on ring C were evident, the surplus substituents (one aldehyde group, two methoxy groups) should be located at ring B. The HMBC correlations of aldehyde proton signal, δ_H 10.22 with C-7 (δ_C 159.1), C-8 (δ_C 115.6), C-9 (δ_C 152.1) suggested the placement of the formyl group at C-8. Two methoxy groups located at C-6 and C-7 was also supported by the HMBC correlation of the methoxy proton signals δ_H 3.80 with C-6 (δ_C 147.2) and δ_H 3.82 with C-7 (δ_C 159.1). Thus, the structure of **1** was established as shown in Fig. 2.

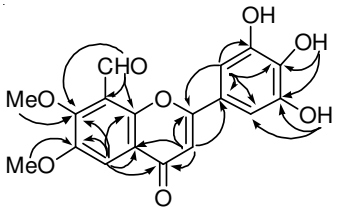

Fig. 2. Selected HMBC (⌒) correlations of compound **1**

TABLE-1
^1H NMR AND ^{13}C NMR DATA (C_5D_5N) OF COMPOUNDS **1**

No.	Compound **1**	
	δ_C (mult.)	δ_H (mult, *J*, Hz)
2	164.8 s	
3	106.2 d	6.68 s
4	178.5 s	
5	122.8 d	
6	147.2 s	
7	159.1 s	
8	115.6 s	
9	152.1 s	
10	121.3 s	
1'	123.1 s	
2',6'	106.8 d	7.06, s
3',5'	147.5 s	
4'	138.2 s	
-CHO	192.6 s	10.22 s
-OMe-6	55.8 q	3.80 s
-OMe-7	61.8 q	3.82 s
-OH-3',5'		10.92 brs
Ar-OH-4'		11.26 brs

Since the flavonoids exhibited strong antioxidant activities[15,16] the antioxidant activity of compound **1** was determined by the detection of the oxidative products with the 2',7'-dichlorofluorescin diacetate (DCFH) method reported previously[6]. Compound **1** shows antioxidant activity with an

IC_{50} value of 2.78 mg/mL. This indicated that compound **1** is high antioxidant activity.

Since some of the flavonoids exhibited anti virus activities[15,17,18] compound **1** was tested for their potencies in preventing anti-tobacco mosaic virus activity using the half-leaf method[7].

The antiviral inhibition rates of the compound at the concentration of 20 μM tested by the half-leaf method. The results showed that the compound **1** exhibited inhibition activities against tobacco mosaic virus replication with inhibition rate of 85.7 %. This rate is higher than that of the positive control, ningnanmycin (34.8%).

N. glutinosa were pretreated with solutions of compounds or a solution of DMSO for 6 h before inoculation with tobacco mosaic virus. At the concentration of 20 μM, compound **1** showed potent protective effects to the host plants, with the inhibition rates ranging from 80.4-88.9 %. The results indicated that pretreatment with the compound **1** can increase the resistance of the host plant to tobacco mosaic virus infection.

ACKNOWLEDGEMENTS

This work was supported by program for Innovative Research Team (in Science and Technology) in University of Yunnan Province (IRTSTYN) and Green Chemistry and Functional Materials Research for Yunnan Research Team (2011HC008).

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