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Flavonoids from *Cleistocalyx operculatus*

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Abstract

Two flavonoids 3′-formyl-4′,6′-dihydroxy-2′-methoxy-5′-methylchalcone and (2S)-8-formyl-5-hydroxy-7-methoxy-6-methylflavanone together with five known compounds, were isolated from the dried buds of *Cleistocalyx operculatus*. Their structures were determined on the basis of spectroscopic analyses (UV, IR, EIMS, ^{1}H, ^{13}C NMR and HMBC).

Keywords: *Cleistocalyx operculatus*; Myrtaceae; Flavonoids; Chalcones; Flavanones

1. Introduction

Cleistocalyx operculatus (Roxb.) Merr. et Perry (Myrtaceae), is a well known medicinal plant whose buds are commonly used as an ingredient for tonic drinks in Southern China. Previous phytochemical attention has led to the characterization of oleanane-type triterpene from its bark (Nomura et al., 1993), and flavonoids and triterpene acids (Zhang et al., 1990) from its buds. Analysis of its leaf oil by GC and GC/MS has also been reported (Dung et al., 1994). In this study, two new flavonoids 3′-formyl-4′,6′-dihydroxy-2′-methoxy-5′-methylchalcone (**1**) and (2S)-8-formyl-5-hydroxy-7-methoxy-6-methylflavanone (**2**), are reported.

2. Results and discussion

The petroleum ether extract (R_p) on repeated column chromatography yielded two new compounds **1** and **2** and the known compounds β-sitosterol, 8-formyl-5,7-dihydroxy-6-methylflavanone and 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone. The ethyl acetate extract (R_E) afforded two compounds 7-hydroxy-5-methoxy-6,8-dimethylflavanone and ursolic acid. The identification of the known compounds was accomplished by comparing their UV, IR, EI MS, ^{1}H and ^{13}C NMR data with those in the literature (Wu et al., 1997; Malterud et al., 1977; Wright et al., 1978; Zhang et al., 1990; Mitscher et al., 1973; Pant and Rastogi, 1977; Seo et al., 1975).

Compound **1** was obtained as orange-yellow needles. The EI MS gave a molecular peak at m/z 312, corresponding to the molecular formula $C_{18}H_{16}O_5$, supported also by elemental analysis (see Section 3). The ^{1}H NMR spectrum indicated the presence of three-proton and two-proton multiplets at δ 7.43 and δ 7.53, respectively, which are typical of a flavonoid nucleus with an unsubstituted B ring. An AB spin system ($J = 16$ Hz) at δ 7.86 and 7.96 suggested the presence of protons of an α,β-unsaturated ketone moiety. The above data and UV spectrum (λ_{max}) at 321 and 283 nm suggested a chalcone nature for compound **1**.

In the HMBC spectrum, the doublet at δ_C 192.5 showed cross peaks to the proton at δ_H 10.1 axis. This one-bond correlation was taken as proof of an aldehyde group. The aldehyde group was confirmed by analysis of the IR spectrum (2870, 2760 cm^{-1}). Additionally, the ^{1}H NMR spectrum showed a one methyl singlet at δ 2.08, one methoxy singlet at δ 3.90 and two hydroxyl group singlets at δ 12.6 and 13.9. These two low-field hydroxyl protons provided supporting evidence for the presence of a formyl group at C-3′, because the downfield shift of OH-4′ (δ_H 12.6) can be explained by hydrogen-bonding with the oxygen atom of a formyl substituent at a neighboring carbon atom, while the downfield shift of OH-6′ (δ_H 13.9) is caused by chelation with the C=O of the α,β-unsaturated keto functionality.

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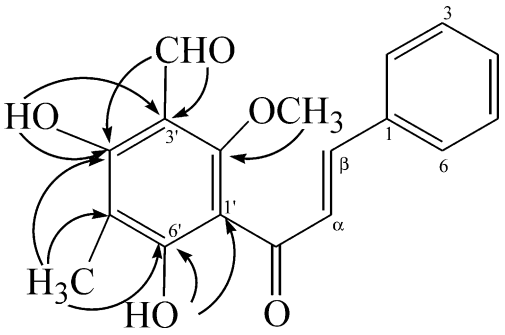

Fig. 1. Selected HMBC of compound **1** (from H to C).

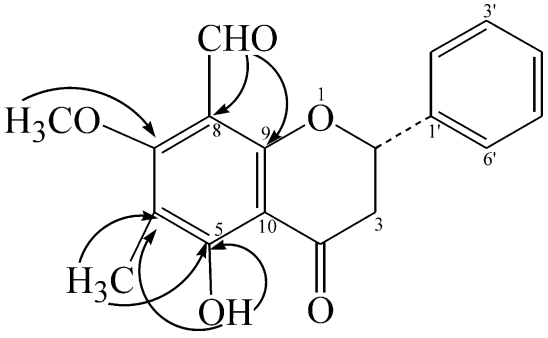

Fig. 2. Selected HMBC of compound **2** (from H to C).

The attachment positions were determined by HMBC experiments (Fig. 1), hence defining compound **1** as 3′-formyl-4′,6′-dihydroxy-2′-methoxy-5′-methylchalcone.

Compound **2** was obtained as yellow needles. The EI MS spectrum gave a molecular ion peak at *m*/*z* 312, corresponding to the molecular formula $C_{18}H_{16}O_5$, supported also by elemental analysis (see Section 3). In the ^{1}H NMR spectrum, the coupled three- and two-proton multiplets were evident at δ 7.45 and δ 7.60, respectively. These signals are typical of a flavonoid nucleus with an unsubstituted B ring. Three one-proton coupled double doublets at *δ* 5.50 and *δ* 2.8–3.2 suggested that ring C was saturated. This splitting pattern was due to the coupling between the H-2 axial proton and the H-3 geminal protons. The above data and UV absorptions (λ_{max}) at 267 nm and 335 nm (*sh*) suggested a flavanone nature for compound **2**.

In the HMBC spectrum, the doublet at δ_C 195.1 showed cross peaks to the proton at δ_H 10.2 axis. This one-bond correlation was taken as proof of an aldehyde group. The aldehyde group was confirmed by analysis of the IR experiments (2870, 2770 cm^{-1}). Additionally, the ^{1}H NMR spectrum showed one methyl singlet at *δ* 2.08, one methoxy singlet at *δ* 3.90 and one hydrogen-bonded hydroxy singlet at δ 12.70. Finally, the attachment positions were determined by HMBC experiments (Fig. 2). The levorotatory nature of the compound indicated S stereochemistry at C-2. From the above evidence, the structure of **2** was determined as (2*S*)-8-formyl-5-hydroxy-7-methoxy-6-methylflavanone.

3. Experimental

3.1. General experimental procedures

Melting points were determined on an XT4A micro-melting apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer Polarimeter 341. UV spectra were recorded on a Varian Cary 500 UV/vis spectrophotometer. IR spectra were obtained on a Nicolet Magna-IR550 infrared spectrophotometer. EIMS were obtained with a Micromass GCT instrument at 70 eV ionization energy. ^{1}H NMR, ^{13}C NMR and 2D NMR spectra were recorded on a Brüker DRX 500 spectrometer (^{1}H 500 MHz and ^{13}C 125 MHz) in $CDCl_3$, with TMS as internal standard. Elemental analysis data were obtained with a Elementar Vario EL III. Known compounds were identified by comparison of their spectral data with those in the literature and, when available, with authentic samples. Silica gel (200–300 mesh) for CC and GF_{254} for analytical TLC were from the Qingdao Marine Chemical Factory, China. Sephadex LH-20 for CC was from Pharmacia Biotech AB, Uppsala Sweden.

3.2. Plant material

Buds of *C. operculatus* were collected in Guangzhou, Guangdong province, China, in May 2002, where the plant is widely cultivated. A voucher specimen (NO. 20025), identified by Associate Professor Dr. Z.N. Gong, is deposited at Institute of Biochemistry, East China University of Science and Technology, Shanghai, 200237, China.

3.3. Extraction and isolation

The air-dried buds (4.0 kg) of *C. operculatus* were extracted with MeOH–H_2O (7:3, 10L×3) at room temp., with the combined extracts evaporated in vacuo. The residue was suspended in H_2O, and then extracted with petroleum ether and EtOAc at room temp. After removal of solvent in vacuo, the following residues were sequentially obtained: petroleum ether extract, R_P (18.4 g) and and ethyl acetate extract, R_E (397.8 g).

R_P was subjected to silica gel chromatography, eluted with petroleum ether–EtOAc (gradient from 40:1 to 5:1). According to the differences in composition monitored by TLC (GF_{254}) 20 fractions (1–20) were obtained. Fraction 4 was fractionated on a Sephadex LH-20 column eluting with MeOH. Five fractions (A_1–E_1) were obtained. After crystallization of fraction E_1 from MeOH, 3′-formyl-4′,6′-dihydroxy-2′-methoxy-5′-methylchalcone (30 mg) (**1**) was obtained. Fraction 6 was fractionated with a Sephadex LH-20 column eluting with MeOH, to give fractions (A_2–E_2). Fraction E_2 gave 8-formyl-5, 7-dihydroxyl-6-methylflavanone (18 mg)

after crystallization from MeOH. Fraction 18 was also fractionated with a Sephadex LH-20 column eluting with MeOH, from which six fractions (A_3–F_3) were obtained. Fraction F_3 gave (2*S*)-8-formyl-5-hydroxy-7-methoxyl-6-methylflavanone (40 mg) (**2**) after crystallization from MeOH.

Fraction 14 was further subjected to Sephadex LH-20 chromatography with MeOH as eluent, to afford six fractions (A_4–F_4). After crystallization of fraction E_4 from MeOH, 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (0.604 g) was obtained. Fraction 15 was also further subjected to Sephadex LH-20 chromatography with MeOH as eluent. Six fractions (A_5–F_5) were obtained. Crystallization of fraction F_5 from MeOH gave β-sitosterol (12.5 mg).

R_E was applied to a silica gel column, eluted with a $CHCl_3$–MeOH (gradient from 25:1 to 2:1). According to differences in composition monitored by TLC (GF_{254}), six fractions (A–F) were obtained. Fraction A was fractionated on a Sephadex LH-20 column eluting with MeOH yielding six fractions (A_6–F_6). Fraction E_6 gave 7-hydroxy-5-methoxy-6,8-dimethylflavanone (56 mg) after crystallization from MeOH. Fraction E was also fractionated on a Sephadex LH-20 column eluting with MeOH. Six fractions (A_7–F_7) were obtained. After crystallization from MeOH fraction F_7 gave ursolic acid (1.3 g), which was the main constituent in the buds of *C. operculatus*.

3.4. 3′-Formyl-4′,6′-dihydroxy-2′-methoxy-5′-methylchalcone (*1*)

Orange yellow needles (MeOH), mp 123–124 °C; UV (MeOH) λ_{max} nm (log ε): 283 (4.28), 321 (4.35); IR (KBr) γ_{max} 3450, 2870, 2760 (*w*), 1625, 1550, 1450, 770, 700 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; EI MS [M^+] 312 (100), 311 (54), 235 (59), 209 (20), 208 (20), 207 (9), 103 (16); Elemental analysis: found: C 69.18%, H 5.18%, requires: C 69.22%, H 5.16%.

3.5. (2S)-8-Formyl-5-hydroxy-7-methoxy-6-methylflavanone (*2*)

Yellow needles (MeOH), mp 154–155 °C; $[\alpha]_D^{25}$ −2.4° (MeOH, C = 0.01); UV λ_{max} (MeOH) nm (log ε): 267 (4.21), 335 (3.80, *sh*); IR (KBr) γ_{max} 3450, 2870, 2770 (*w*), 1690, 1630, 1590, 1460, 770, 700 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; EI MS [M^+] 312 (100), 311 (33), 235 (37), 208 (82), 180 (66), 104 (12); Elemental analysis: found: C 69.17%, H 5.19%, requires: C 69.22%, H 5.16%.

Table 1
^1H and ^{13}C NMR spectral data for compounds 1 and 2, *J* (Hz) in parentheses

Proton	1	2	Carbon	1	2
β	7.96, *d*, (16)		β	147.0	
α	7.86, *d*, (16)		α	125.0	
			CO	193.0	
			1′	108.2	138.0
2′		7.60, *m*	2′	167.1	127.0
3′		7.45, *m*	3′	108.5	130.0
4′		7.45, *m*	4′	165.7	130.0
5′		7.45, *m*	5′	109.3	130.0
6′		7.60, *m*	6′	169.0	127.0
			1	136.0	
2	7.43, *m*	5.50, *dd*, (12.8, 2.9)	2	128.7	80.0
3	7.53, *m*	2.82eq, *dd*, (16.9, 2.9) 3.12ax, *dd*, (16.9, 12.8)	3	129.2	41.0
4	7.53, *m*		4	132.1	188.0
5	7.53, *m*		5	129.2	166.8
6	7.43, *m*		6	128.7	110.0
			7		166.2
			8		110.8
			9		167.0
			10		108.5
CHO	10.10, *s*	10.20, *s*	CHO	192.5	195.1
4′-OH	12.60, *s*				
5-OH		12.70, *s*			
6′-OH	13.90, *s*				
OMe	3.90, *s*	3.90, *s*	OMe	67.0	65.0
Me	2.08, *s*	2.08, *s*	Me	8.1	8.0

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