· Letters ·

A New Phenylated Flavone from *Melicope pteleifolia*

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Abstract: **Objective** To study the constituents in *Melicope pteleifolia*. **Methods** Plant material was isolated with 80% EtOH. Compounds were separated with chromatographic methods and their structures were elucidated on the basis of spectral analysis (EI-MS, ^{1}H-NMR, and ^{13}C-NMR) and chemical evidence. **Results** Five compounds were isolated from petrol ether or ethyl acetate soluble fraction. Their structures were identified as 3,5,3'-trihydroxy-4'-methoxy-7-(3-methylbut-2-enyloxy) flavone (pteleifolosin C, **1**), 3,7-dimethoxyl kaempferol (kamatakenin, **2**), vanillic acid (**3**), tricosanoic acid tetradecyl ester (**4**), and β-sitosterol (**5**), respectively. **Conclusion** Compound **1** is a new structure named pteleifolosin C. Compounds **2**－**4** are isolated from this plant for the first time.

Key words: kamatakenin; *Melicope pteleifolia*; tricosanoic acid tetradecyl ester; 3,5,3'-trihydroxy-4'-methoxy-7-(3-methylbut-2-enyloxy) flavone; vanillic acid

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Introduction

Melicope pteleifolia (Champ. ex Benth.) Hartley was ever placed in genus *Euodia* J. R. et G Forst with scientific name *Euodia lepta* (Spreng.) Merr. In *Flora of China* Volume 11 published in 2008, most species described in *Euodia* J. R. et G Forst are revised and reclassified to genus *Melicope* J. R. et G Forst or *Tetradium* Loureiro (Jiangsu Medical College, 2008). In the southern area of China and in the neighboring district of Southeast Asia, *M. pteleifolia* is a medicinal herb and an edible plant (Gunawardana *et al*, 1987; Shaari *et al*, 2006). Besides used as a staple material of Guangdong herbal tea, it also serves to treat injury, wounds, fester, and eczema (Pharmacopoeia Committee of P. R. China, 1977) and widely applies in many Chinese patent medicines. There are few chemical researches on *M. pteleifolia* (Li *et al*, 2010). In our research on new bioactive natural products, we have investigated the leaves of *M. pteleifolia* collected from Conghua (Guangdong, China), which led to the isolation of five compounds, including a new flavonol (pteleifolosin C, **1**) and four known compounds, namely kamatakenin (**2**), vanillic acid (**3**), tricosanoic acid tetradecyl ester (**4**), and β-sitosterol (**5**). In this paper, we present the isolation and structure elucidation of these compounds.

Materials and methods

General experimental procedures

NMR spectra of compounds **1**－**4** were obtained on Bruker Avance 500 MHz and Bruker DRX—400 spectrometer respectively with the undertreated residual signal of the solvent as internal standard. EI-MS was obtained with a Shimadzu QP5050A (GC-MS) Spectrometer at 70 eV. Column chromatography (CC) and thin layer chromatography (TLC) were performed on silica gel H from Qingdao Haiyang Chemical Co., Ltd. Preparative HPLC column was Sinochrom ODS-BP (250 mm × 10 mm, 5 μm) from Dalian Elite Analytical Instruments Co., Ltd. Gel filtration was performed on Pharmacia Sephadex LH-20.

Plant material

The leaves of *Melicope pteleifolia* (Champ. ex Benth.) were collected in June, 2008, Conghua (Guangdong,

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China) and identified by Prof. PAN Chao-mei. A voucher specimen was deposited at the Laboratory of Phytochemistry, Guangzhou University of Chinese Medicine (Guandong, China).

Extraction and separation

The dried leaves powder (5 kg) of *M. pteleifolia* was isolated with 80% EtOH to yield crude extract which was fractionated in a Soxhlet apparatus to obtain petrol ether (PE) (60—90 ℃), ethyl acetate, acetone, and methanol soluble fractions successively. The PE fraction was subjected to CC over silica gel using solvents of increasing polarity. The eluate obtained with 25% EtOAc in PE was subsequently subjected to gel filtration (Sephadex LH-20) eluting with $CHCl_3$-MeOH (1:1) to give yellow powder, which was purified by preparative HPLC and yielded compound **1** (25 mg). The eluate with 15% EtOAc in PE gave compounds **4** (43 mg) and **5** (1.3 g) after being filtrated with Sephadex LH-20. The part of ethyl acetate fraction (300 g) was subjected to CC over silica gel (MeOH-$CHCl_3$) and gel filtration on Sephadex LH-20 to give compounds **2** (19 mg) and **3** (33 mg).

Results and discussion

Compound **1**: amorphous yellow powder, mp 182—184 ℃, soluble in pyridine and dimethyl sulfoxide, less soluble in methanol and acetone, and insoluble in chloroform and PE. Reaction with magnesium and hydrochloric acid gave violet-red color suggesting a flavone core (Fig. 1).

Protons (20) and carbons (21) signals were shown in ^{1}H-NMR and ^{13}C-NMR spectra (Table 1) and $[M]^+$ ion at *m/z* 384 given by EI-MS specified the molecular formula as $C_{21}H_{20}O_7$. ^{13}C-NMR and DEPT showed three primary carbons, one second carbon, six tertiary carbons, and eleven quaternary carbons. Thus, three protons are not attached to carbon, i.e, they are active hydrogens.

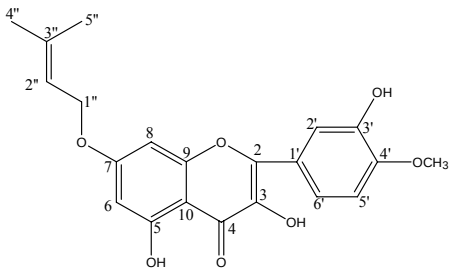

Fig. 1 Structure of compound 1

The ^{1}H-NMR spectrum revealed resonances for a methoxyl group at δ 3.85 (3H, s) and a phenyloxy substituent at δ 1.76 (3H, s), 1.73 (3H, s), 5.46 (1H, t, J = 6.5 Hz), and 4.64 (2H, d, J = 6.5 Hz) (Ahsan *et al*, 1994; Amaral *et al*, 2001). The base peak at *m/z* 316 in mass spectrum due to the loss of $[C_5H_8]^+$ from molecular ion agreed with the existence of phenyloxy group (Cong and Li, 2003). Therefore, there are five substituents, namely, three hydroxyl groups, one methoxyl group, and one phenyloxy group, attached to flavone nucleus.

The ABX spin-system at δ 7.72 (1H, d, J = 1.6 Hz, H-2'), 7.09 (1H, d, J = 8.5 Hz, H-5'), and 7.68 (1H, dd, J = 1.6, 8.5 Hz, H-6') was shown in ^{1}H-NMR spectrum (Table 1), and indicated the presence of a 3',4'-disubstituted B-ring. The HMBC correlations between the methoxyl proton at δ 3.85 (3H, s), 7.68 (H-6'), and 7.72 (H-2') (Fig. 2) and the carbon at δ 149.4 indicated that the methoxyl group was located at C-4'. Similarly,

Table 1 NMR data of compound 1 in DMSO-d_6 (^{1}H-NMR: 500 MHz, ^{13}C-NMR: 125 MHz)

Position	δ_C	δ_H	Position	δ_C	δ_H
2	146.7 (s)		3'	146.2 (s)	9.31 (1H, s, -OH)
3	136.4 (s)	9.55 (1H, s, -OH)	4'	149.4 (s)	
4	176.0 (s)		5'	111.8 (d)	7.09 (1H, d, J = 8.5 Hz)
5	160.3 (s)	12.43 (1H, s, -OH)	6'	119.7 (d)	7.68 (1H, dd, J = 1.6, 8.5 Hz)
6	97.9 (d)	6.33 (1H, d, J = 2.0 Hz)	1"	65.3 (t)	4.64 (2H, d, J = 6.5 Hz)
7	164.1 (s)		2"	119.0 (d)	5.46 (1H, t, J = 6.5 Hz)
8	92.5 (d)	6.72 (1H, d, J = 2.0 Hz)	3"	138.1 (s)	
9	156.0 (s)		4"	25.3 (q)	1.76 (3H, s)
10	103.9 (s)		5"	18.0 (q)	1.73 (3H, s)
1'	123.3 (s)		-OCH_3	55.6 (q)	3.85 (3H, s)
2'	114.7 (d)	7.72 (1H, d, J = 1.6 Hz)			

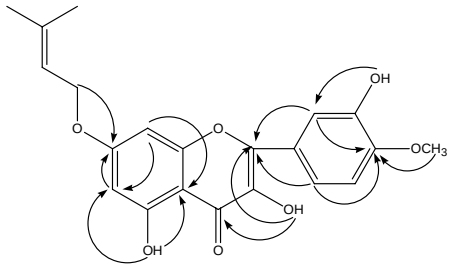

Fig. 2 Significant HMBC correlations of compound 1 (H→C)

the HMBC correlations from the hydroxyl proton (δ 9.31) to C-2' (δ 114.7), C-3' (δ 146.2), and C-4' suggested that the hydroxyl group must be linked to C-3'.

^{1}H-NMR spectrum showed the presence of a hydroxyl at δ 12.43 (readily identified as 5-OH) and a *meta*-coupling system between δ 6.33 (1H, d, J = 2.0 Hz) and 6.72 (1H, d, J = 2.0 Hz), and suggested the 5,7-disubstituted A-ring. C-6 was recognized by the HMBC correlation from 5-OH to the tertiary carbon (δ 97.9). Based on the attribution of C-6 and H-6 (δ 6.33) which was revealed by cross peak in HSQC spectrum, it was unambiguous to distinguish C-7 (δ 164.1) and C-8 (δ 92.5) from C-10 and C-5 (also identified by H-5) with the help of HMBC correlations. The phenyloxy substituent assigned to C-7 can be further confirmed by a ^3J coupling between H-1" and C-7.

The absence of singlet proton signal in the aromatic region clarified a substitute at C-3. In addition, the exhibition of a ^3J correlation between the third hydroxyl proton (δ 9.31) and C-4 revealed that the hydroxyl group was located at C-3. Therefore, compound **1** was identified as 3,5,3'-trihydroxy-4'-methoxy-7-(3-methylbut-2-enyloxy) flavone. It is a new compound named pteleifolosin C.

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References

Ahsan M, Armstronga JA, Gibbons S, Gray AI, Waterman PG, 1994. Novel *O*-prenylated flavonoids from two varieties of *Boronia coerulescens*. *Phytochemistry* 37(1): 256-266.

Amaral DF, Arruda MS, Arruda AC, Müller AH, Pantoja LL, da S. Lima TM, 2001. Flavones from the leaves of *Ficus gomelleira*. *J Brazilian Chem Soc* 12(4): 538-541.

Cong PZ, Li SY, 2003. *Natrual Organic Mass Spectrometry*. China Medical Science Press: Beijing.

Gunawardana YAGP, Cordell GA, Ruangrungsi N, Chomya S, Tantivatana P, 1987. Traditional medicinal plants of Thailand (VII): Alkaloids of *Evodia lepta* and *Evodia gracilis*. *J Sci Soc Thailand* 13: 107-112.

Jiangsu New Medical College, 2008. *Flora of China*.

Li SG, Yang Y, Ye WC, Jiang RW, 2010. Chemical constituents from stems of *Melicope pteleifolia*. *Chin Tradit Herb Drugs* 41(7): 1052-1056.

Pharmacopoeia Committee of P. R. China, 1977. *Pharmacopoeia of People's Republic of China*. Chemical Industry Publishers: Beijing.

Shaari K, Safri S, Abas F Lajis NH, Israf DA, 2006. A geranylacetophenone from the leaves of *Melicope pteleifolia*. *Nat Prod Res* 20(5): 415-419.