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TWO NEW FLAVONES FROM *UVARIA MACROPHYLLA* ROXB. VAR. *MICROCARPA* AND THEIR CYTOTOXIC ACTIVITIES

Zhong Chen, Xue-Run Liu, Yan-Li Liu, Qiong-Ming Xu,* and Shi-Lin Yang

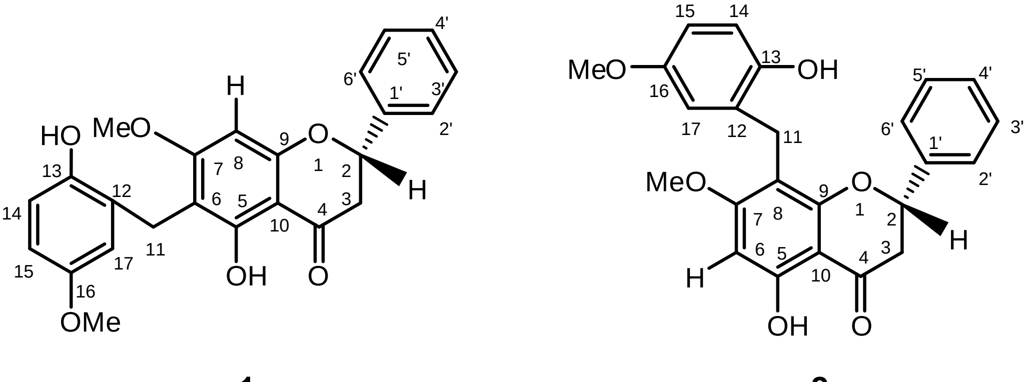
College of Pharmaceutical Sciences, Soochow University, Suzhou, 215123, P. R. China Phone: +86-512-69561421; Fax: +86-512-65882089; E-mail: xuqiongming@suda.edu.cn

Abstract – Two new flavones, macrophyllains **A** and **B**, were isolated from the leaves of *Uvaria macrophylla* Roxb. var. *microcarpa*, and their structures were established on the basis of their spectral data. The two new compounds showed some cytotoxic activity by MTT assay.

Uvaria macrophylla Roxb. var. *microcarpa* is a tropical plant, widely distributed in Hainan, Guangdong and Guangxi provinces of southern China.[1] The leaves of *Uvaria macrophylla* Roxb var. *microcarpa* are also well-known as an important traditional Chinese medicine used for treatment of rheumatic arthritis, functional dyspepsia, chronic pain, cancers and cat fever in folklore.[2] Previous studies on constituents from the *Uvaria* genus led to the isolation of different compounds such as polyoxygenated cyclohexenes, annonaceous acetogenins, alkaloids, flavones.[3] In the course of our investigation for bioactive agents from *Uvaria macrophylla* Roxb. var. *microcarpa*, two new flavones, macrophyllains **A** (**1**) and **B** (**2**), together with four known compounds, myricetin (**3**),[4] chrysin (**4**),[5] kaempferol (**5**),[6] and quercetin (**6**),[7] were obtained from its chloroform extracts. We now report the isolation and structure elucidation of the new compounds and their cytotoxic activities.

Macrophyllain A (1) was isolated as yellow oil. The molecular formula was determined to be $C_{24}H_{22}O_6$ by high resolution (HR) - electron ionization (EI) - mass spectrometry. The IR spectrum of compound **1** suggested the presence of hydroxyl group(s) (3487, 3434 cm^{-1}), carbonyl group(s) (strong absorption at 1652 cm^{-1}) and aromatic moiety (1611, 1504 cm^{-1}). Its UV spectrum exhibited characteristic absorptions for dihydroflavone [λ_{max} (logε): 288 (4.65) and 207 (5.01) nm].[8] The ^{1}H-NMR spectrum of compound **1**

showed the presence of a phenolic hydroxyl at *δ* 12.12 (s) ascribed to 5-OH. The signals at *δ* 7.4-7.5 (m, 5H) were assigned to the protons of ring B. The signals at *δ* 5.44 (dd, 1H, *J* = 13.0, 3.0 Hz), 3.12 (dd, 1H, *J* = 17.5, 13.0 Hz) and 2.82 (dd, 1H, *J* = 17.5, 3.0 Hz) consisting of ABX system were ascribed to H-2, H-3α and H-3β respectively. The signals of another simplified ABX system at *δ* 6.78 (d, 1H, *J* = 3.0 Hz), 6.62 (dd, 1H, *J* = 8.5, 3.0 Hz) and 6.72 (d, 1H, *J* = 8.5 Hz) were ascribed to the protons of ring D. The ^{13}C- and DEPT NMR spectra revealed 24 carbon signals including two primary, two secondary, ten tertiary and ten quarternary carbons. Observation of the heteronuclear multiple bond connectivity (HMBC) correlations (Figure 1) of the protons at *δ* 3.78 (s, 2H) with the carbons at δ 163.1, 164.4, 148.2, 116.6, 126.3 and 107.3 suggested that a 2-hydroxy-5-methoxybenzyl group was linked to C-6 position, similarly the two methoxyl at δ 3.95 (s, 3H) and 3.51 (s, 3H) were determined to be located at C-7 and C-16 position respectively. The proposed structure of compound **1** was verified by the nuclear overhauser effect spectroscopy (NOESY) spectrum (Figure. 2). The circular dichroism (CD) spectrum of compound **1** exhibited a split curve centered at λ 318 nm with a positive cotton effect at λ 335 (+5.86) and a negative cotton effect at λ 302 (-7.42), thus the absolute configuration of C-2 was established as S.[9]

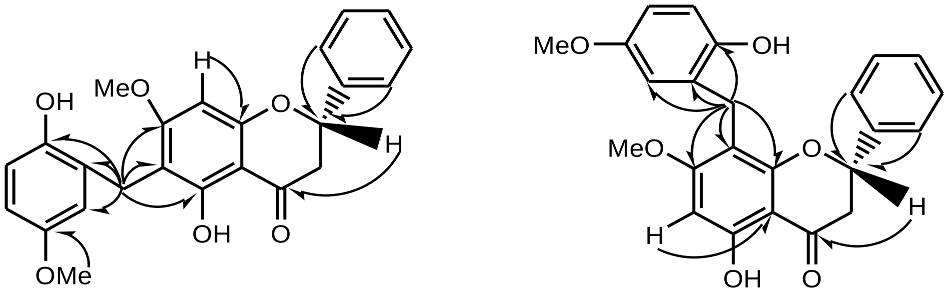


1 **2**

Scheme 1

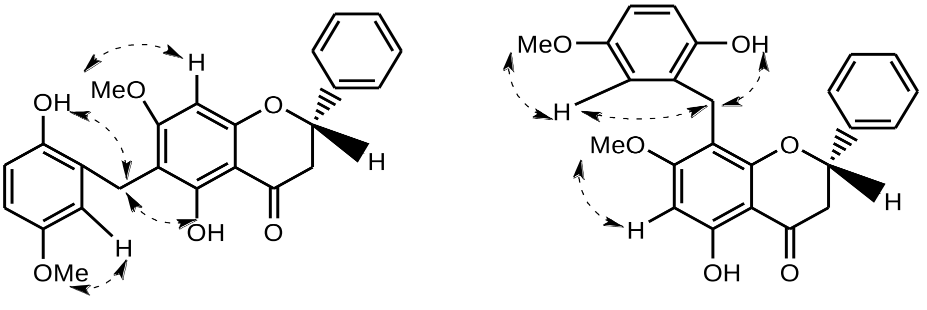
Macrophyllain B (2) was obtained as yellow oil. The molecular formula was determined to be $C_{24}H_{22}O_6$ by HR-EI-MS. Compound **2** had similar IR and UV spectra to those of compound **1**. In comparison the EI-MS, ^{1}H-NMR and ^{13}C-NMR spectra data with those of compound **1**, compound **2** was postulated to be a structural isomer of compound **1**. In the HMBC experiment (Figure 1), H-C (11) at *δ* 3.82 (s, 2H) showed correlations with C-9 at *δ* 159.0, C-7 at *δ* 165.2, C-8 at *δ* 108.9, C-13 at *δ* 148.4, C-17 at *δ* 117.0, and C-12 at *δ* 126.9, which suggested that 2-hydroxy-5-methoxybenzyl group was linked to C-8 position. The proposed structure of compound **2** was verified by the NOESY spectrum (Figure 2). The CD spectrum of compound **2** exhibited a split curve centered at λ 316 nm with a positive cotton effect at λ 333 (+3.92) and a negative cotton effect at λ 301 (-9.15), thus the absolute configuration of C-2was established as S.[9]

Cytotoxic activities of compounds **1, 2, 3, 4, 5** and **6** were determined by MTT assay and expressed as IC_{50}, with norcantharidin as a positive control. The IC_{50} values of compounds **1**, **2**, **3**, **4**, **5**, **6** and norcantharidin for A549 lung carcinoma cell were 37.26, 41.74, 26.15, 18.55, 34.29, 25.48, and 0.71 μg/mL respectively, for SK-MES-1 lung carcinoma cell those were 39.29, 39.77, 23.98, 16.66, 37.81, 27.26 and 0.96 μg/mL, and for NCI-446 lung carcinoma cell those were 36.52, 37.96, 24.75, 16.98, 39.72, 27.08 and 0.50 μg/mL. Our results suggested that flavones might be, at least in part, responsible for the proposed therapeutic effect of leaves of *Uvaria macrophylla* Roxb. *var. microcarpa*.



1 2

Figure 1. Key HMBC correlations of compounds **1** and **2**



1 2

Figure 2. Key NOESY correlations of compounds **1** and **2**

EXPERIMENTAL

General

Melting points were determined by the XT5 micro-melting-point apparatus (XT5, Beijing families instrument light instrument plant, China) and are uncorrected. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. IR spectra were taken on a Perkin-Elmer 983 G spectrometer. ^{1}H-, ^{13}C-NMR and 2D NMR spectra were recorded on a Varian Inova 500 spectrometer in $CDCl_3$ using using tetramethylsilane (TMS) as internal standard.. HR-ESI-MS spectra were determined on a Micromass Q-TOF2 spectrometer. CD spectra were determined on a JASCO-715 spectrometer. Semi-preparative HPLC was carried out on a column of ODS (250 mm × 9.4 mm i.d., Agilent Zorbax SB-C_{18}, Palo Alto, USA) with a Waters 2996 detector, the flow rate was 2 mL/min and the wavelength for detection was 254 nm. Medium pressure liquid chromatography (MPLC) was carried out on a column of silica gel H (460 mm × 26 mm i.d., Büchi Borosilikat 4.6, Flawil, Swiss). Sephadex LH-20 was obtained from GE Healthcare Bio-Science AB. Silica gel (200-300 mesh) for column chromatography was obtained from Qingdao Marine Chemical Factory, Qingdao, China. Precoated plates of silica gel used for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, China. Compounds on the TLC were detected under UV. A549 lung carcinoma cell, SK-MES-1 lung carcinoma cell and NCI-446 lungcarcinoma cell were purchased from Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Plant material

The leaves of *Uvaria macrophylla* Roxb. var. *microcarpa.*were collected in Danzhou City Hainan province of China in May 2009, and identified by Professor Chunyu Liu. A voucher sample (No. 01-05-27-09) was deposited in the Herbarium of the College of Pharmaceutical Sciences, Soochow University.

Extraction and Isolation

The dried plant material (5.0 kg) was extracted with 90% EtOH (60 L) three times under reflux, and the solvent was subsequently removed under reduced pressure to give the EtOH extract (0.85 kg), which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble fraction (31.5 g) was further portioned between petroleum ether and 90% MeOH (V/V). The petroleum ether fraction (13.2 g) was chromatographed over silica gel column (5.0 × 60.0 cm), which was eluted with petroleum ether/EtOAc (0-100%) to afford 10 fractions. Fraction 3 was subjected to semi-preparative RP-HPLC with 80%

MeCN to give macrophyllain A **1** (4.8 mg, yield 0.000096%) and macrophyllain B **2** (7.5 mg, yield 0.00015%). Fraction 5 was further purified by MPLC on polyamides (100-200 mesh), with a $CHCl_3$/MeOH gradient eluents (the ratios of $CHCl_3$/MeOH were from 9:1 to 8:2), yielded pure myricetin **3** (25 mg, yield 0.00050%), and pure chrysin **4** (12 mg, yield 0.00024%). Fraction 7 was chromatographed over Sephadex LH-20 column (4.0 × 60.0 cm), which was eluted with $CHCl_3$/MeOH (5:5) to afford kaempferol (**5**, 345 mg, yield 0.00690%) and quercetin (**6**, 2.3 g, yield 0.04600%).

Macrophyllain A (1) yellow oil, $[\alpha]_D^{20}$ -64.16 (c 0.03, MeOH). UV λ_{max} (MeOH) nm: 288, 207. IR (KBr) ν_{max} cm^{-1}: 3487, 3434, 2935, 2912, 2834, 1652, 1611, 1504, 1460, 1372, 1342, 1268, 1232, 1178, 1074, 1040, 955, 867, 829, 772, 705. HR-EI-MS m/z: 406.1412 ($[M]^+$, calcd. for $C_{24}H_{22}O_6$, 406.1416). EI-MS m/z: 406 (100), 270 (92), 255 (25), 166 (85), 151 (48) 138 (5), 105 (21), 77 (12), 69 (36). CD: 1.25 (λ 230.5 nm), +2.72 (λ 256.0 nm), -4.66 (λ 280.5 nm), -7.42 (λ 302.0 nm), -6.95 (λ 312.5 nm), -5.10 (λ 315.5 nm), -2.04 (λ 318.0 nm), +2.92 (λ 321.5 nm), +3.75 (λ 329.5 nm), +5.86 (λ 335.0 nm), +4.69 (λ 338.5 nm), +3.25 (λ 350.0 nm). ^{1}H and ^{13}C-NMR data see Table 1.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of compounds **1** and **2** ($CDCl_3$, δ ppm)

	1		**2**	
	δ_C	δ_H	δ_C	δ_H
2	79.8	5.44 dd (13.0, 3.0*)	79.5	5.39 dd (13.0, 3.0)
3	43.4	3.12 dd (17.5, 13.0) 2.82 dd (17.5, 3.0)	43.2	3.08 dd (17.5, 13.0) 2.83 dd (17.5, 3.0)
4	196.2		196.1	
5	163.1		165.2	
6	107.3		91.9	6.13 s
7	164.4		165.2	
8	93.1	6.15 s	108.9	
9	158.9		159.0	
10	103.3		102.9	
11	23.5	3.78 s	22.7	3.82 s
12	126.3		126.9	
13	148.2		148.4	
14	116.6	6.72 d (8.5)	116.9	6.79 d (8.5)
15	113.4	6.62 dd (8.5, 3.0)	112.8	6.65 dd (8.5, 3.0)
16	153.0		153.0	
17	116.4	6.78 d (3.0)	117.0	
1′	138.0		138.1	
2′	126.3	7.4-7.5 m	126.1	7.4-7.5 m

3′	129.0	7.4-7.5 m	128.9	7.4-7.5 m
4′	129.2	7.4-7.5 m	129.0	7.4-7.5 m
5′	129.0	7.4-7.5 m	128.9	7.4-7.5 m
6′	126.3	7.4-7.5 m	126.1	7.4-7.5 m
7-OCH_3	56.3	3.95 s	56.2	3.91 s
16-OCH_3	55.4	3.51 s	55.7	3.74 s
5-OH		12.12 brs		12.78 brs
13-OH		6.34 brs		6.79 brs

* Data in parentheses are *J* values (in Hz).

Macrophyllain B (2) yellow oil, $[\alpha]_D^{20}$ +4.16 (c 0.08, MeOH). UV λ_{max} (MeOH) nm: 295, 212. IR (KBr) ν_{max} cm^{-1}: 3466, 3428, 2932, 2910, 2836, 1650, 1608, 1500, 1465, 1375, 1340, 1268, 1230, 1179, 1075, 1042, 950, 865, 826, 770, 708. HR-EI-MS m/z: 406.1410 ($[M]^+$, calcd. for $C_{24}H_{22}O_6$, 406.1416). EI-MS m/z: 406 (85), 270 (100), 166 (45), 151 (28) 138 (8), 105 (11), 77 (12), 69 (18). CD: +0.18 (λ 226.5 nm), +3.45 (λ 255.5 nm), -3.05 (λ 279.0 nm), -9.15 (λ 301.0 nm), -6.47 (λ 310.5 nm), -4.29 (λ 314.0 nm), -1.68 (λ 316.0 nm), +1.85 (λ 322.5 nm), +3.09 (λ 328.5 nm), +3.71 (λ 331.0 nm), +3.92 (λ 333.0 nm), +3.43 (λ 350.0 nm). ^1H and ^{13}C-NMR data see Table 1.

Cytotoxic activity

To evaluate the cytotoxic activities of the flavones from *Uvaria macrophylla* Roxb. var. *microcarpa* against human A549, SK-MES-1 and NCI-446 tumor cell lines, the MTT colorimetric assay had been performed. The amount of formazan was determined by photometer at 570 nm. Cells were plated into 96-well flat-bottomed cultured plates at a concentration of 5×10^4 cells per well in complete RPMI 1640 culture medium. Twenty-four hours after plating, the medium containing foetal calf serum was removed and test solutions were given to cells in various final concentrations such as 2.5, 5, 10, 20, 50, 100 μg/mL. After incubation with drugs for 24 h, MTT solution was added to the wells and plates were incubated at 37 ºC for 4 h. Results were expressed as percentage of the absorbance in control cells compared to that in the drug-treated cells.

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