HETEROCYCLES, Vol. 89, No. 2, 2014, pp. 465 - 471. © 2014 The Japan Institute of Heterocyclic Chemistry
Received, 16th November, 2014, Accepted, 17th December, 2013, Published online, 24th December, 2013
DOI: 10.3987/COM-13-12887

TWO NEW LINEAR FURANOCOUMARINS FROM *ANGELICA APAENSIS*

Yong Li,[b] Min Zhang,[b] Min Luo,[a] Xue Wu,[a] Qiuli Xiao,[a] Juan Hua,[a] and Xian Li[a]*

[a] School of Pharmacy, Kunming Medical University, Kunming 650031, Yunnan, P.R. China
(*Corresponding author: phone: +86-871-65922780; fax: +86-871-65922780; e-mail: xianlikm@163.com)
[b] The 5th Affiliated Hospital, Kunming Medical University, Gejiu 661000, Yunnan, P.R. China

Abstract –Two new linear furanocoumarins, together with 13 known analogues, were isolated from the roots of *Angelica apaensis*. The structures of new compounds were elucidated by spectroscopic methods including extensive 1D and 2D NMR experiments, as well as high-resolution EI mass analysis. All furanocoumarins obtained were assayed for their cytotoxic ability against K562 and SMMC-7721 human tumor cell lines.

The genus *Angelica*, which belongs to the family Umbelliferae, includes more than 60 species of medicinal herbs. From various phytochemical studies, it has been now established that majority of *Angelica* species comprise high amounts of biologically active coumarins with antiproliferative, antitumour and anticancer, analgesic and anti-inflammatory, acetylcholinesterase inhibitory, antiatherogenesis, antimicrobial and antiplatelet activities.[1,2] *Angelica apaensis*, which belongs to the family Umbelliferae, is a perennial herb widely distributed in the northeast of Yunnan, China. The roots of this plant have been used extensively as folk medicines to treat cough, asthma, wind colic, tympanites and flatulency.[3,4] With an aim to access bioactive constituents from this plant, the present study on *A. apaensis*, which was collected in Qiaojia County of Yunnan Province, China, afforded two new linear furanocoumarins (**1** and **2**), along with 13 known analogues, cnidilin (**3**),[5] sen-byakangelicole (**4**),[6] isobyakangelicol (**5**),[4] byakangelicol (**6**),[7] 5-(2-hydroxy-3-methoxy-3-methylbutoxy)soralen (**7**),[7]

9-(2-hydroxy-3-methoxy-3-methylbutoxy)ergapten (**8**),[7] byakangelicin (**9**),[8] isobyakangelicin (**10**),[9] 5,8-di(2,3-dihydroxy-3-methylbutoxy)psoralen (**11**),[10] marmesin (**12**),[11] 7-methoxycoumarin (**13**),[12] scopoletin (**14**),[10] hamaudol (**15**).[8] In this study, we describe the isolation and structure determination of two new linear furanocoumarins (**1** and **2**), and their cytotoxic activity against K562 and SMMC-7721 human tumor cell lines.

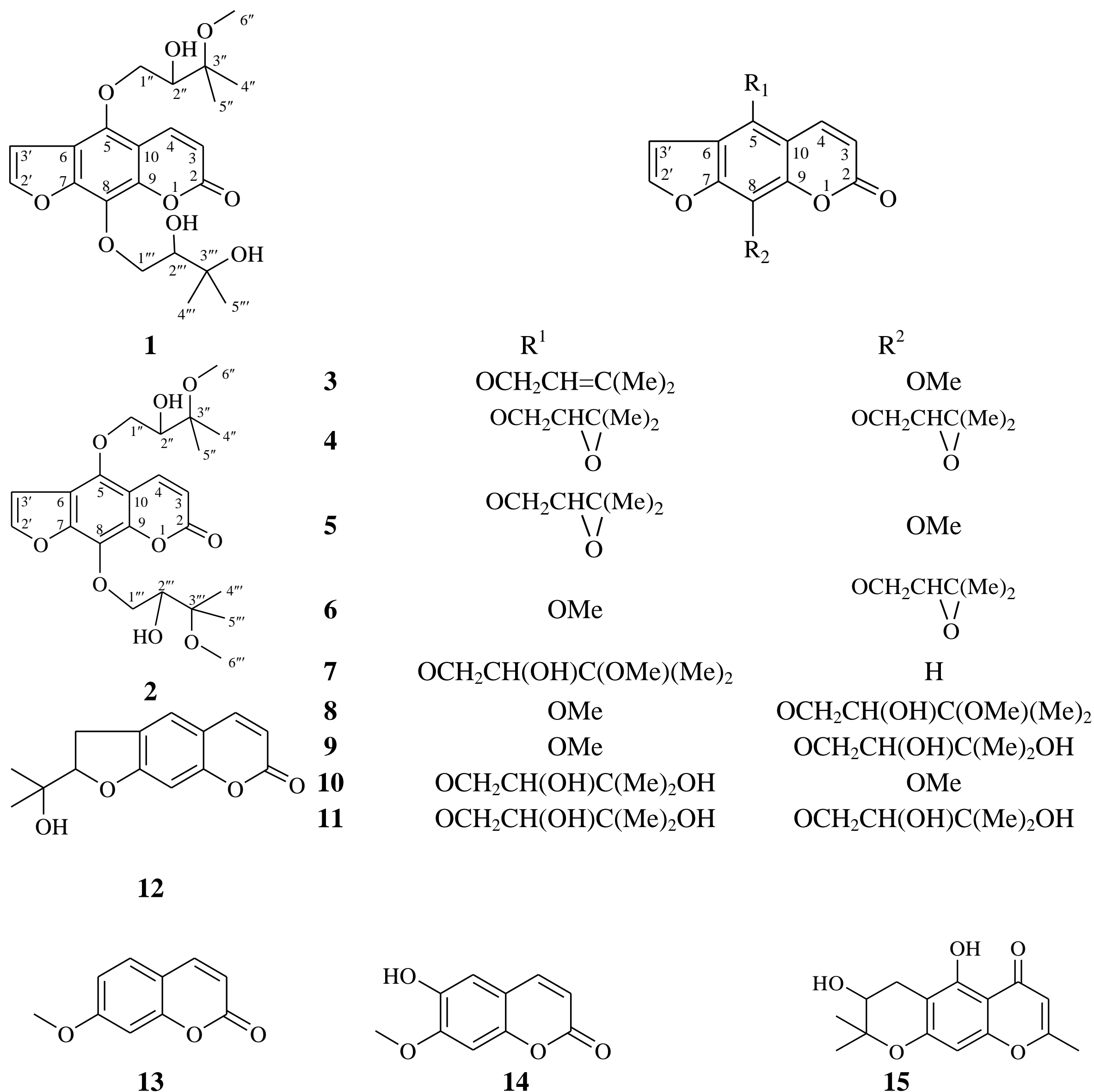

Chart 1

Compound (**1**) was obtained as pale yellow amorphous solid with a quasi-molecular ion peak at *m*/*z* 436 $[M]^+$ in its positive-ion EI-MS, corresponding to molecular formula of $C_{22}H_{28}O_9$, which was verified by HR-EIMS spectrum (found: 436.1740; calcd: 436.1733). Its strong yellowish green fluorescence under UV light (268 nm) and the signals of the aromatic region in its ^1H and ^{13}C NMR spectra were typical of

furocoumarins.[13] In the ^{1}H NMR spectrum of **1**, two doublet signals (1H, J = 9.8 Hz) at δ 8.33 and 6.24 were assigned as the protons of pyrone ring. Two doublet signals (1H, J = 2.3 Hz) at δ 7.89 and 7.23 were determined the protons of furan ring.[10] The ^{1}H NMR showed the presence of 5,8-disubstituted furanocoumarin moiety, a 2″-hydroxy-3″-methoxy-3″-methylbutoxy moiety [δ 4.67 (1H, dd, J = 9.9, 2.2 Hz, H-1″), 4.27 (1H, m, overlap, H-1″), 3.92 (1H, dd, J = 7.6, 1.4 Hz, H-2″), 3.21 (3H, s, H-6″), 1.24 (3H, s, H-5″), 1.18 (3H, s, H-4″)], and a 2‴,3‴-dihydroxy-3‴-methylbutoxy moiety [δ 4.59 (1H, dd, J = 10.1, 2.9 Hz, H-1‴), 4.27 (1H, m, overlap, H-1‴), 3.85 (1H, dd, J = 8.0, 2.9 Hz, H-2‴), 1.23 (3H, s, H-5‴), 1.21 (3H, s, H-4‴)]. The position of the 2″-hydroxy-3″-methoxy-3″-methylbutoxy moiety was established by both a ^{1}H-^{13}C long-range correlation between H-1″ (δ 4.67 and 4.27) with C-5 (δ 144.6) in the HMBC spectrum of **1** (Figure 1) and a positive NOE effect between H-3′ (δ 7.23) and H-1″. The 2‴,3‴-dihydroxy-3‴-methylbutoxy moiety was deduced to be attached to C-8 based on the HMBC correlation between H-1‴ (δ 4.59) to C-8 (δ 128.1). Full assignments were established by 2D NMR including ^{1}H-^{1}H COSY, HSQC, HMBC, ROESY experiments. Therefore, Compound **1** was identified as 5-[2-hydroxy-3-methoxy-3-methylbutoxy]-8-[2,3-dihydroxy-3-methylbutoxy]psoralen.

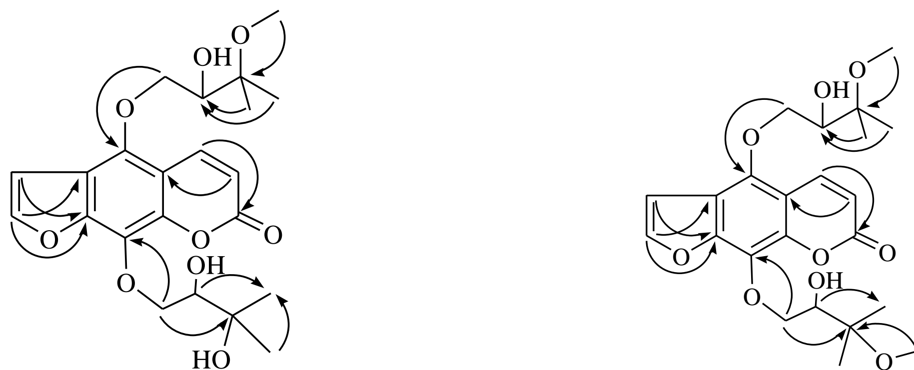

Figure 1. The selected HMBC correlations of compounds **(1)** and **(2)**

Compound (**2**), also obtained as pale yellow amorphous solid, exhibited a molecular ion peak at m/z 450 $[M]^+$ in its positive-ion EI-MS, in combination with the ^{1}H and ^{13}C NMR spectra and HR-EIMS spectrum (found: 450.1895; calcd: 450.1890), established a molecular formula of $C_{23}H_{30}O_9$. A general analysis of the NMR data of **2** showed that it resembled compound **1** very much except for the substituent of C-3‴. The difference can be rationalized as the hydroxy group at C-3‴ in **1** replaced by a methoxy (C-6‴ δ 49.2) in **2**. The ^{13}C NMR signal of C-3‴ at δ 77.6 which is 6.1 ppm lower than that of free byakagelicin, suggested that methoxy moiety was linked to C-3‴ position of **2**.[14] The location of methoxy moiety was established by the HMBC long-range correlation between C-3‴ and H-6‴ (δ 3.31) (Figure 1). Full assignments were established by 2D NMR including ^{1}H-^{1}H COSY, HSQC, HMBC, ROESY experiments. Thus, compound **2** was determined to be 5,8-di-(2-hydroxy-3-methoxy-3-methylbutoxy)psoralen.

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds (**1** and **2**)

	1		**2**	
	δ_C	δ_H (mult; J, Hz)	δ_C	δ_H (mult; J, Hz)
2	160.4 (s)		162.7 (s)	
3	113.2 (d)	6.24 (d, 9.8)	113.2 (d)	6.31 (d, 9.7)
4	140.8 (d)	8.33 (d, 9.8)	141.9 (d)	8.40 (d, 9.7)
5	144.6 (s)		144.8 (s)	
6	116.6 (s)		117.6 (s)	
7	150.8 (s)		151.4 (s)	
8	128.1 (s)		128.9 (s)	
9	145.0 (s)		145.4 (s)	
10	109.0 (s)		109.7 (s)	
2′	146.6 (d)	7.89 (d, 2.3)	147.1 (d)	7.84 (br s)
3′	106.2 (d)	7.23 (d, 2.3)	106.3 (d)	7.18 (br s)
1″	76.7 (t)	H_a: 4.67 (dd, 9.9, 2.2)	76.8 (t)	H_a: 4.63 (br d, 10.1)
		H_b: 4.27 (m, overlap)		H_b: 4.26 (m, overlap)
2″	77.6 (d)	3.92 (dd, 7.6, 1.4)	76.9 (d)	3.95 (br d, 8.2)
3″	77.0 (s)		77.7 (s)	
4″	19.9 (q)	1.18 (s)	20.2 (q)	1.20 (s, overlap)
5″	26.8 (q)	1.24 (s)	22.4 (q)	1.26 (s, overlap)
6″	49.5 (q)	3.21 (s)	49.2 (q)	3.31 (s, overlap)
1‴	76.4 (t)	H_a: 4.59 (dd, 10.1, 2.9)	76.5 (t)	H_a: 4.55 (br d, 10.2)
		H_b: 4.27 (m, overlap)		H_b: 4.26 (m, overlap)
2‴	77.6 (d)	3.85 (dd, 8.0, 2.9)	76.9 (d)	3.87 (br d, 8.3)
3‴	71.8 (s)		77.7 (s)	
4‴	22.3 (q)	1.21 (s)	20.6 (q)	1.20 (s, overlap)
5‴	25.5 (q)	1.23 (s)	22.2 (q)	1.26 (s, overlap)
6‴			49.2 (q)	3.31 (s, overlap)

* Assignments were based on the analysis of DEPT, HSQC, HMBC and ROESY spectra.

Compounds (**3**–**15**) were known compounds, whose structures were elucidated by comparisons with the literature.

Compounds (**1**–**15**) were tested for cytotoxicity against human-tumor K562 and SMMC-7721 cell lines, with fluorouracil as the positive control. Compound **4** exhibited weak cytotoxic inhibition with the IC_{50} values of 43.97 μg/mL and 38.06 μg/mL, respectively. Other compounds showed noncytotoxicity with IC_{50} values of > 100 μg/mL. Although compound **3** has an active center of dimethylallyl group,[15] this compound was completely inactive which suggested that the substituents in the molecules also affected its cytotoxicity.

EXPERIMENTAL

General. Column chromatography (CC): silica gel (200–300 mesh). TLC: silica gel *GF_{254}*; Melting points were obtained on an XRC-1 apparatus, and uncorrected. Optical rotations: SEPA-300 polarimeter. IR spectra: BIO-RAD FTS-135 spectrometer with KBr pellets; ν_{max} in cm^{-1}. UV Spectra: Shimadzu 210A double-beam spectrophotometer; λ_{max} log (ε) in nm. ^{1}H- and ^{13}C-NMR spectra: Bruker AM-400 and DRX-500 spectrometer with acetone-d_6 as solvent and TMS as internal standard. MS: VG Auto Spec-3000 magnetic sector instrument; *m/z* (rel. %).

Plant Material. The roots of *Angelica apaensis* were collected in Qiaojia, Yunnan Province, P.R. China, in October 2011. The identity of the plant material was verified by Prof. Qi-Tai Zhang. A voucher specimen (KIB-2011-66 Zhang) was deposited in State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, P.R. China.

Extraction and Isolation. The air-dried and powdered roots parts of *A. apaensi* (6.0 kg) were extracted three times with MeOH at room temperature for 24 h and filtered. The filtrate was evaporated and the resulting residue partitioned between H_2O and EtOAc. The EtOAc fraction (165.0 g) was subjected to column chromatography over a silica gel (200–300 mesh, 2.5 kg) column, eluting with $CHCl_3$/Me_2CO (1:0–0:1 gradient system) to give 5 fractions L_1–L_5. Fraction L_1 was subjected to silica gel column chromatography, eluting with petroleum ether/EtOAc (from 9:1 to 6:4), Sephadex LH-20 (Me_2CO) and recrystallization to yield **1** (6.0 mg), **2** (8.0 mg), **4** (15.0 mg), **5** (6.0 mg), **6** (21.0 mg), **8** (11.0 mg), and **11** (5.0 mg). Fractions L_2 were submitted to repeated chromatography over silica gel with petroleum ether/ Me_2CO (9:1), Sephadex LH-20 (Me_2CO), MCI–gel (60–100% MeOH–H_2O, MeOH, Me_2CO) and recrystallization to yield **7** (25.0 mg), **10** (6.0 mg) **12** (18.0 mg), and **14** (10.0 mg). Fraction L_3 was subjected to chromatography over silica gel with petroleum ether/2-propanol (20:1), MCI–gel (60–100% MeOH–H_2O, MeOH, Me_2CO) and recrystallization to yield **3** (10.0 mg), **9** (6.0 mg), **13** (12.0 mg), and **15** (9.0 mg).

5-(2-Hydroxy-3-methoxy-3-methylbutoxy)-8-(2,3-dihydroxy-3-methylbutoxy)psoralen (**1**): pale yellow amorphous solid, mp 124–126 °C; $[\alpha]_D^{19.3}$ -0.59 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε): 221 (4.44) nm; IR (KBr) ν_{max} cm^{-1}: 3474, 3440, 2973, 1705, 1592, 1480, 1460, 1438, 1364, 1352, 1315, 1152, 1094, 1055; EIMS (70 eV) *m/z* (rel. int.): 436 $[M]^+$ (18), 334 (13), 219 (12), 218 (82), 217 (24), 73 (100), 59 (52); HR-EIMS *m/z*: 436.1740 $[M]^+$ (calcd. for $C_{22}H_{28}O_9$, 436.1733); ^{1}H and ^{13}C NMR: see Table 1.

5,8-Di-(2-hydroxy-3-methoxy-3-methylbutoxy)psoralen (**2**): pale yellow amorphous solid, mp 126–1286 °C; $[\alpha]_D^{19.3}$ +1.72 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε): 222 (4.42) nm; IR (KBr) ν_{max} cm^{-1}: 3447, 2975, 1728, 1593, 1479, 1437, 1362, 1353, 1324, 1204, 1147, 1089, 1061; EIMS (70 eV) *m/z* (rel. int.): 450 $[M]^+$ (11), 334 (21), 219 (10), 218 (77), 217 (18), 73 (100); HR-EI-MS *m/z*: 450.1895 $[M]^+$ (calcd.

for $C_{23}H_{30}O_9$, 450.1890); ^1H and ^{13}C NMR: see Table 1.

Cytotoxicity Bioassay. Cytotoxicity of compounds against suspended tumor cells was determined by the trypan blue exclusion method, and against adherent cells by sulforhodamine B (SRB) assay. Cells were plated in a 96-well plate 24 h before treatment and continuously exposed to different concentrations (100, 10, 1, and 0.1 μM) of compounds for 72 h. After compound treatments, cells were counted (suspended cells) or fixed and stained with SRB (adherent cells) as described in the literature.[16] The assays were performed in triplicate, on separate occasions. The IC_{50} values were calculated by the *Logit* method. The percentage of inhibition was calculated according to the inhibiton(%) = $[(OD_0–OD_1)/OD_0] \times 100$ formula, where OD_0 is the optical density of control, OD_1 is that of the sample.

ACKNOWLEDGEMENTS

Financial support of this research was provided by the Natural Science Foundation of Yunnan Province (No. 2011FB058), and the National Natural Science Foundation of China (No. 21102060).

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