Pergamon

PII: S0031-9422(96)00634-6

Phytochemistry, Vol. 44, No. 5, pp. 887–889, 1997

0031-9422/97 $17.00+0.00

ANTIMICROBIAL CONSTITUENTS OF *ANGELICA DAHURICA* ROOTS

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(*Received in revised form* 12 *August* 1996)

Key Word Index—*Angelica dahurica*; Umbelliferae; roots; coumarins; 5,8-di(2,3-dihydroxy-3-methylbutoxy)-psoralen; ferulic acid; antimicrobial activity.

Abstract—One novel coumarin from *Angelica dahurica* roots was elucidated to be 5,8-di(2,3-dihydroxy-3-methylbutoxy)-psoralen. It occurs together with six other known coumarins and ferulic acid. The antimicrobial activity of the coumarins and ferulic acid were compared. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The dried root of *Angelica dahurica* (Umbelliferae) is an important oriental medicinal preparation [1–2]. To date, over twenty coumarins have been isolated from this crude drug [3–6]. This paper deals with structure elucidation and antimicrobial activities of one novel and six known coumarins.

RESULTS AND DISCUSSION

The EtOAc-soluble fraction of the MeOH extract of the dried roots was subjected to column chromatography. Bioassay-guided isolation provided eight antimicrobial constituents (**1**–**8**). The antimicrobial activities were tested against fungi and bacteria (Table 1).

The UV spectrum of **1** showed the characteristic features of a 5,8-dioxygenated psoralen skeleton [7] and the ^{1}H-NMR spectrum was almost identical to sen-byak-angelicole [3] except for the presence of a hydroxyl. Two pairs of AB doublets [δ 8.36, 1H, *d*, $J = 9.77$; 6.25, 1H, *d*, $J = 9.77$; 7.9, 1H, *d*, J = 2.44; 7.22, 1H, *d*, $J = 2.44$] indicated a 5,8-dioxygenated psoralen, and two pairs of AMX splitting patterns [δ 4.74, 1H, *dd*, $J = 9.77$, 2.44; 4.31, 1H, *dd*, $J = 9.77$, 8.55; 3.84, 1H, *m*; 4.59, 1H, *dd*, $J = 9.77$, 3.2; 4.28, 1H, *dd*, $J = 9.77$, 7.94; 3.87, 1H, m] and two pairs of geminal dimethyl signals [δ 1.27, 1.25 and δ 1.23, 1.22] suggested the presence of C-5 and C-8 disubstitued 2,3-dihydroxy-3-methylbutoxyl moieties attached to the psoralen skeleton [8–9]. The ABX in the lower field was ascribable to one of the side chains attached at C-8 [10–11]. The presence of a hydroxyl was also confirmed by the IR spectrum (3400 cm^{-1}) and the MS spectrum [dehydrated fragments: 405$(M+H-H_2O)^+$, 387$(M+H-2H_2O)^+$]. Thus **1** is a novel furanocoumarin, namely 5,8-di(2,3-dihydroxy-3-methyl-butoxy)-psoralen.

1 could be produced as an artefact from sen-byak-angelicole [3] by hydrolysis of the corresponding bis-epoxide by hot MeOH. We therefore extracted directly with EtOAc at room temperature which was carefully concentrated *in vacuo* under 40°. When this extract was applied to a silica gel TLC plate, developed with benzene:acetone:methanol (6:3:1) the presence of **1** was apparent at R_f 0.48. The known constituents (see Experimental) were identified by spectral comparison with literature data.

EXPERIMENTAL

^{1}H-NMR spectra were recorded using deuterized solvents as the internal standards.

Plant material. The roots of *A. dahurica* were collected from the cultivated land around Jinbu, Kang Won Province in 1994.

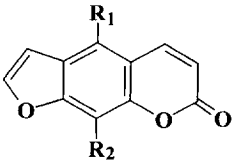
Extraction and isolation. The dried and chopped roots (20 kg) were refluxed with MeOH (4 hr × 3) on a water bath.

The extracts were combined and concentrated *in vacuo* to afford a residue, which was suspended in H_2O and successively partitioned with benzene, EtOAc and then BuOH.

The EtOAc fraction (6 g) was subjected to silica-gel column chromatography (Wakogel C-100) using stepwise elution with hexane–EtOAc–MeOH from hexane 100% to MeOH 100%. Each fraction was monitored by TLC and subjected to the antimicrobial assays.

Table 1. Antimicrobial activity of coumarins and ferulic acid from the EtOAc fraction of *Angelica dahurica*

	MIC (μ/ml)							
Test organism	1	2	3	4	5	6	7	8
Bacillus subtilis	>1000	>500	62.5	>1000	500	250	250	250
Escherichia coli	>1000	1000	>1000	>500	1000	>1000	>1000	>250
Cladosporium herbarum	>500	62.5	>1000	>1000	>1000	>1000	62.5	250
Aspergillus candidus	>62.5	125	250	>1000	>1000	250	>125	>125



1. R_1=—OCH_2-CH(OH)—$C(CH_3)_2OH$
R_2=—OCH_2-CH(OH)—$C(CH_3)_2OH$

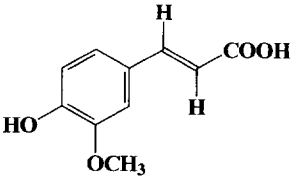
3. R_1= H
R_2=—OCH_2-CH(OH)—$C(CH_3)_2OH$

4. R_1=—OCH_2-CH=$C(CH_3)_2$
R_2= H

5. R_1= H
R_2=—OCH_2-CH=$C(CH_3)_2$

6. R_1=—OCH_3
R_2=—OCH_2-CH=$C(CH_3)_2$

7. R_1=—OCH_3
R_2=—OCH_2-CH(OH)—$C(CH_3)_2OH$



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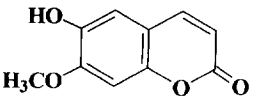

8

The active fractions **1**–**8** were further purified by silica gel column chromatography (Wakogel C-300) to give 5,8-di(2,3-dihydroxy-3-methylbutoxy)-psoralen (**1**), ferulic acid (**2**), (*R*)-heraclenol (**3**), isoimperatorin (**4**), imperatorin (**5**), phellopterin (**6**), byakangelicin (**7**) and scopoletin (**8**), using linear gradient elution by toluene to toluene–EtOAc (2:1), toluene to toluene–acetone (4:1).

Compound **1** $[\alpha]_D^{30} = +34$ (MeOH, $c = 0.1$); UV λ_{max}^{MeOH} nm (log ε) 218 (2.03), 242 (2.68), 249 (2.69), 267 (2.73), 312 (2.79); IR ν_{max}^{KBr} cm^{-1}; 3400 (OH), 1708 (C=O), 1593, 1478, 1409 (C=C); ^{1}H-NMR (500 MHz, acetone-d_6, ppm); 8.36 (1H, *d*, *J* = 9.77, H-4), 7.9 (1H, *d*, *J* = 2.44, H-2′), 7.22 (1H, *d*, *J* = 2.44, H-3′), 6.25 (1H, *d*, *J* = 9.77, H-3), 4.74 (1H, *dd J* = 9.77, 2.44, H-1″), 4.59 (1H, *dd*, *J* = 9.77, 3.22, H-1‴), 4.31 (1H, *dd*, *J* = 9.77, 8.55, H-1″a), 4.28 (1H, *dd*, *J* = 9.77, 7.94, H-1‴a), 3.87 (1H, *m*, H-2″a), 3.84 (1H, *m*, H-2‴a), 1.27 (3H, s, Me), 1.25 (3H, s, Me), 1.23 (3H, s, Me), 1.22 (3H, s, Me) ^{13}C-NMR (125 MHz, acetone-d_6, ppm); 160.7 (C-2), 151.1 (C-7), 146.9 (C-5), 145.4 (C-2′), 145.1 (C-8a), 141.1 (C-4), 128.5 (C-8), 116.9 (C-6), 113.6 (C-3), 109.4 (C-4a), 106.6 (C-3′), 78.1 (C-2″ or C-2‴) 77.9 (C-2″ or C-2‴), 77.1 (C-1″ or C-1‴ 76.9 (C-1″ or C-1‴), 72.15 (C-3″ or C-3‴), 72.13 (C-3″ or C-3‴), 27.3 (C-4″ or C-4‴), 27.1 (C-4″ or C-4‴), 25.9 (C-5″ or C-5‴), 25.6 (C-5″ or C-5‴). EI-MS *m/z* (rel. int.); 423 (M+H, 53.1)$^+$, 405 (M+H-H_2O, 100)$^+$, 387 (M+H-2H_2O, 30.3)$^+$, 303 (M+H-2,3-dihydroxy-3-methylbutoxy, 25)$^+$.

Antimicrobial assay. The MIC value against bacteria and fungi were determined by the serial 2-fold dilution method. Bacteria were pre-cultured in 10 ml of nutrient-broth medium for 12 h at 27° on a shaker, and then diluted 100-fold with the same medium. Fungi were inoculated into 10 ml of a potato-malt extract-sucrose agar medium, and incubated at 27° for 7 days to form a well-extended fungal mat with spores, these spores being collected by filtration. The spores were suspended in 50 ml of a medium containing 0.2% glucose, 0.1% yeast extract, 0.1% citric acid, and 0.37% $Na_2HPO_4 12H_2O$. Liquid culture or spore-suspension cultures containing the best compound were placed in the wells of a 96-well microplate at 27° for 24 h. The growth of the bacteria was evaluated by the degree of turbidity of the culture with the naked eye, and the spore germination was examined under a microscope.

Acknowledgement—We thank the SC-NMR laboratory of Okayama University for the 500 MHz experiment.

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