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BIOLOGICALLY ACTIVE PHENOLIC CONSTITUENTS FROM *ALPINIA GAGNEPAINII* K. SCHUM. (ZINGIBERACEAE)

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SUMMARY

*The fractionation of the MeOH extract from the rhizomes of Alpinia gagnepainii K. Schum. (Zingiberaceae) collected in province Lai Chau, Vietnam, was directed by the antibacterial activity against Gram-positive and Gram-negative bacteria as well as the free radical scavenging activity of the soluble fractions of the MeOH extract. β-Sitosterol, stigmasterol, cardamomin (**2**), 5,6-dehydrokawain (**4**), naringenin 5-O-methyl ether (**5**), and a new phenolic compound, designated alpininone (**3**), were isolated. Their structures were determined on the basis of spectroscopic analyses. (–)-Pinocembrine (**1**), which was isolated from A. gagnepainii K. Schum. collected in province Quang Binh, Vietnam, **2**, and **3** showed a good correlation with the antibacterial activity of the soluble fractions, while **4** was selective against fungi. However **1**-**5** did not demonstrate the free radical scavenging activity in the DPPH assay.*

Key words: *Alpinia gagnepainii*; Zingiberaceae; phenolic constituent; antimicrobial activity; free radical scavenging activity.

I - INTRODUCTION

Previously we reported the isolation of (–)-pinocembrine (**1**), cardamomin (**2**), (–)-epicatechin and 3-*O*-*β*-sitosteryl *β*-*D*-glucopyranoside from *Alpinia gagnepainii* K. Schum. (Vietnamese name: Rieng Gagnepain) growing in province Quang Binh [1]. Further study on *A. gagnepainii* species collected in province Lai Chau resulted in the isolation of **2**, a new phenolic compound **3**, designated alpininone, 5,6-dehydrokawain (**4**), and naringenin 5-*O*-methyl ether (**5**). We present here the isolation and structure determination of the newly isolated compounds from *A. gagnepainii* and the antimicrobial and free radical scavenging activities of the isolates from *A. gagnepainii*.

II - EXPERIMENTAL

General Procedure

^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained on a Bruker Avance 500 spectrometer with tetramethyl silane (TMS) as reference. EI-MS (70 eV) were measured on a Hewlett-Packard 5989B mass spectrometer. ESI-MS was recorded on a LC/MSD Trap Agilent Series 1100 system with a Zobax SB C18 column, using 15% MeOH in (H_2O + formic acid) in 35 min, at a flow rate of 0.4 ml/min. Silica gel (63-100 μm, Merck) was used for open column (CC) and flash column (FC) chromatography. TLC was performed on precoated DC Alufolien 60 F_{254} plates (Merck) and detected by UV light (254 nm) and/or by spraying with 1% vanillin in conc. H_2SO_4 and 5% $FeCl_3$ in EtOH.

Plant Material

The rhizomes of *A. gagnepainii* were collected and identified by Mr. Nguyen Quoc Binh, a botanist of the Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, in July 2005 in Dien Bien, province Lai Chau, Vietnam.

Extraction and Isolation

The fresh rhizomes of *A. gagnepainii* (15 kg) were sliced, air-dried and then oven-dried at 50°C to give a dry material (3 kg). The material was powdered and then extracted with MeOH by percolation at room temperature. The combined MeOH extracts were concentrated under reduced pressure and the residue was subjected to sequential partition between H_2O and organic solvents of increasing polarity, *n*-hexane and EtOAc, successively, to give the corresponding *n*-hexane- (**GH**) (35.6 g, 1.19% yield on the basis of the dry weight) and EtOAc-soluble fraction (**GE**) (54 g, 1.8% yield). Part of the *n*-hexane-soluble fraction (10 g) was repeatedly chromatographed on silica gel CC and FC, using *n*-hexane–$(CH_3)_2CO$ gradient, to give β-sitosterol and stigmasterol as a mixture, alpininone (**3**), and 5,6-dehydrokawain (**4**). Part of the EtOAc-soluble fraction (10 g) was repeatedly chromatographed on silica gel CC [*n*-hexane–$(CH_3)_2CO$ gradient and CH_2Cl_2–$(CH_3)_2CO$ gradient], silica gel FC [*n*-hexane–$(CH_3)_2CO$ gradient], and silica gel FC [CH_2Cl_2–$(CH_3)_2CO$ gradient] to give cardamomin (**2**), **3**, **4**, and **5**.

Alpininone (3): Pale yellow needles. mp. 264 - 265°C. R_f = 0.48 [*n*-hexane–$(CH_3)_2CO$, 2:1]. UV (EtOH) λ_{max}: 235, 325. IR ν_{max} (KBr) cm^{-1}: 3382, 1641, 1599, 1580, 1514, 1466, 1358, 1276, 1167, 976, 838, 650. ^{1}H-NMR ($CDCl_3$): δ 0.88 (3H, t, *J* = 7 Hz, H-10), 1.31 (8H, m, H-6, H-7, H-8, H-9), 1.66 (2H, quintet, *J* = 7 Hz, H-5), 2.63 (2H, t, *J* = 7.5 Hz, H-4), 6.59 (1H, d, *J* = 16 Hz, H-2), 6.87 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 7.42 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 7.50 (1H, d, *J* = 16 Hz, H-1), 8.69 (1H, br s, OH). ^{13}C-NMR ($CDCl_3$): δ 14.1 (q, C-10), 22.6 (t, C-9), 24.7 (t, C-5), 29.1 (t, C-7), 29.4 (t, C-6), 31.7 (t, C-8), 40.7 (t, C-4), 116.2 (d, C-3′, C-5′), 123.4 (d, C-2), 126.2 (s, C-1'), 142.8 (d, C-1), 159.6 (s, C-4′), 201.1 (C-3). EI-MS (70 eV): *m/z* (%) 246 ($C_{16}H_{22}O_2$, $[M]^{+\cdot}$) (4.9), 162 (41.5), 147 (100), 119 (24.6), 107 (17), 91 (20.1). ESI-MS: positive mode 247.1 ($[M+H]^+$), negative mode 245.1 ($[M-H]^-$).

5,6-Dehydrokawain (4): Yellow needles. mp. 136°C. R_f = 0.37 [*n*-hexane–$(CH_3)_2CO$, 2:1]. UV, IR, EI-MS, ^{1}H- ($CDCl_3$), and ^{13}C-NMR ($CDCl_3$) spectroscopic data are comparable with the literature values [2].

Naringenin 5-O-methyl ether (5): Colorless needles. mp. >260°C. $[\alpha]_D^{25}$ = −253.3 (c=0.3, MeOH). IR, EI-MS, ^{1}H- (DMSO-d_6), and ^{13}C-NMR (DMSO-d_6) spectroscopic data are comparable with the literature values [3].

Evaluation of antimicrobial activity and determination of minimum inhibitory concentration (MIC)

The broth microdilution method was used for preliminary evaluation and MIC determination [4]. The strains of bacteria, fungi and yeast were listed in the table 1. **GH** and **GE** are *n*-hexane- and EtOAc-soluble fractions of the MeOH extract of the rhizomes of *A. gagnepainii* growing in province Lai Chau. Compound **1** was isolated in our previous work from *A. gagnepainii* growing in province Quang Binh, compound **2**, **3**, and **4** were isolated in this study from *A. gagnepainii* growing in province Lai Chau.

Evaluation of free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [5] was employed in the evaluation of the free radical scavenging activity of **GH**, **GE**, and compounds **1** - **5**.

III-RESULTS AND DISCUSSION

Extraction, Isolation, and Structure elucidation of compounds 3-5

The dried rhizomes of *A. gagnepainii* were extracted with MeOH, and the resultant MeOH

extract was fractionated into *n*-hexane- (**GH**) and EtOAc-soluble (**GE**) fractions. Compounds **3**, **4**, β-sitosterol, and stigmasterol were isolated from **GH** and **2**-**5** from **GE** by repeated CC and FC.

Compound **3** was isolated as pale yellow needles, mp. 264 - 265°C. Its molecular formula was determined as $C_{16}H_{22}O_2$ from EI-MS (*m/z* 246, $[M]^{+\cdot}$) and ESI-MS spectra (247.1, $[M+H]^+$; 245.1, $[M-H]^-$). The IR spectrum showed hydroxyl absorption at 3382 cm^{-1}, carbonyl absorption at 1641 cm^{-1}, and absorptions of aromatic rings at 1599, 1580, 1514, and 1466 cm^{-1}. The ^1H- and ^{13}C-NMR spectra of **3** showed a disubstituted benzene moiety, in which a hydroxyl group [δ_H 8.69 (br s)] was placed at C-4' [δ_H 6.87 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 7.42 (2H, d, *J* = 8.5 Hz, H-2', H-6')], a *trans*-α, δ-unsaturated carbonyl group [δ_H 6.59 (1H, d, *J* = 16 Hz, H-2), 7.50 (1H, d, *J* = 16 Hz, H-1); δ_C 123.4 (d, C-2), 142.8 (d, C-1), and 201.1 (C-3)], and a long-chain alkyl group [δ_H 0.88 (3H, t, *J*= 7 Hz, H-10), 1.31 (8H, m, H-6, H-7, H-8, H-9), 1.66 (2H, quintet, *J* = 7 Hz, H-5), 2.63 (2H, t, *J* = 7.5 Hz, H-4)]. This alkyl residue was deduced to be attached to the carbonyl group from the ^1H and ^{13}C chemical shifts and coupling constant of H-4 [δ_H 2.63 (2H, t, *J* = 7.5 Hz), δ_C 40.7 (t, C-4)]. Therefore the double bond was in conjugation with the benzene nucleus.

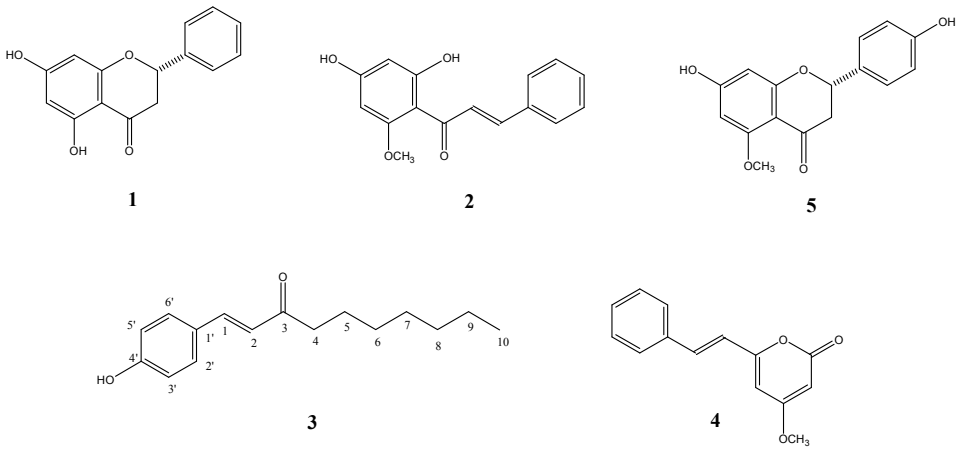

Figure 1: Chemical structures of compounds **1** - **5**

The HMBC spectrum of **3** (Fig. 2) supported the structure of **3** related to compounds of [*n*]-gingerol or [*n*]-shogaol structures, which were isolated from *Zingiber officinale* Roscoe (Zingiberaceae) [3]. Finally, the size of the alkyl chain was determined as seven-carbon from the EI-MS spectrum. Therefore the structure of **3**, a new natural phenolic compound, designated alpininone was determined as shown in Fig. 1.

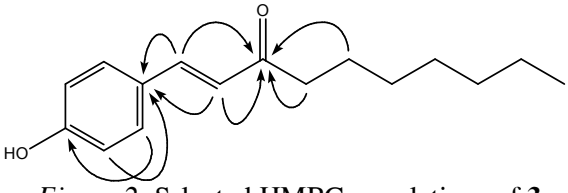

Figure 2: Selected HMBC correlations of **3**

Compound **4** and **5** were determined to be 5,6-dehydrokawain and naringenin-5-*O*-methyl ether by comparing their spectroscopic data (^1H, ^{13}C-NMR, DEPT 90, and DEPT 135 spectra) with those reported in the literature. The negative optical rotation of **5** {$[\alpha]_D^{25}$ −253.3 (c = 0.3, MeOH)} confirmed the stereochemistry at C-2 of **5** as 2*S*.

Evaluation of in-vitro antimicrobial activity

The MICs of **GH**, **GE**, and **1** - **4** were listed in the table 1. They showed both antibacterial activity against Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*, and Gram-positive *Bacillus subtilis* and *Staphylococcus*

aureus. However compounds which may be responsible for the inhibition of *P. aeruginosa* could not be isolated in the present study. The spectrum of activity and the MIC values of compounds **1** - **3** against the bacteria were noticeable. Compound **4** was found to be selective against fungi *Aspergillus niger* and *Fusarium oxysporum*. Interestingly, **1** - **4** represented different structures of phenolic compounds.

Table 1: Antimicrobial activity of the soluble fractions **GH** and **GE** and pure isolates **1**-**4** from *A. gagnepainii*

No.	Sample	Minimum Inhibitory Concentration (MIC, µg/ml)							
		Gram (–) bacterium		Gram (+) bacterium		Fungus		Yeast	
		Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus subtillis*	*Staphylococcus aureus*	*Aspergillus niger*	*Fusarium oxysporum*	*Candida albicans*	*Saccharomyces cerevisiae*
1	**GH**	50	n.a.[a)]	200	n.a.[a)]	n.a.[a)]	n.a.[a)]	n.a.[a)]	n.a.[a)]
2	**GE**	50	50	100	100	n.a.[a)]	n.a.[a)]	200	200
3	**1**	25	n.a.[a)]	50	25	n.a.[a)]	n.a.[a)]	n.a.[a)]	n.a.[a)]
4	**2**	25	n.a.[a)]	50	25	n.a.[a)]	n.a.[a)]	n.a.[a)]	n.a.[a)]
5	**3**	12.5	n.a.[a)]	12.5	12.5	n.a.[a)]	n.a.[a)]	n.a.[a)]	50
6	**4**	n.a.[a)]	n.a.[a)]	n.a.[a)]	n.a.[a)]	50	50	n.a.[a)]	n.a.[a)]

[a)] n.a.: the sample did not show activity in the preliminary test.

Evaluation of free radical scavenging activity

The antioxidant potential of **GH**, **GE**, and **1** - **5** was evaluated using a chemical reaction with a stable radical species 2,2-diphenyl-1-picrylhydrazyl (the DPPH method). As shown in table 2, only the soluble fraction **GE** showed a weak free radical scavenging activity. The pure isolates **1** - **5** were not demonstrated to be free radical scavengers.

Table 2: Free radical scavenging activity of the soluble fractions **GH** and **GE** and pure isolates **1**-**5** from *A. gagnepainii*

No.	Sample	Scavenging activity, %	Scavenging activity, µg/ml
1	Positive reference	87.04±0.0	25.31
2	Negative reference	0.00±0.0	n. a.[a)]
3	**GH**	42.74±2.2	n.a.[a)]
4	**GE**	70.23±1.2	24.99
5	**1**	3.71±0.1	n.a.[a)]
6	**2**	3.28±0.9	n.a.[a)]
7	**3**	11.40±0.7	n.a.[a)]
8	**4**	6.42±0.8	n.a.[a)]
9	**5**	6.17±0.3	n.a.[a)]

[a)] n.a.: the sample did not show the free radical scavenging activity.

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