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Phenolic compounds from the rhizomes of *Zingiber pellitum*

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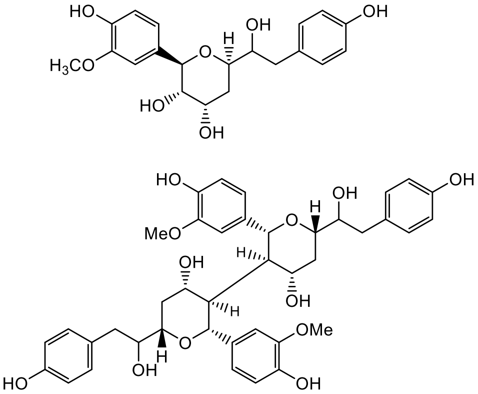
Abstract

Six new phenolic compounds have been isolated from the rhizome of *Zingiber pellitum*, including two new diarylheptanoids, zingiberpyrans A and B, rhamnocitrin-3-*O*-rhamnoside, meranzin hydrate, vanillin and (+)-*α*-viniferin. Their structures were elucidated by analyzing NMR, HRESIMS, and CD spectral evidences combining with the published data. Rhamnocitrin-3-*O*-rhamnoside (**3**) exhibited the strongest inhibition of nitric oxide production in LPS-stimulated RAW264.7 cells (IC_{50} 49.6 µM) while zingiberpyran A (**1**) was less active (IC_{50} 71.0 µM).

Zingiber pellitum Gagnep.



Keywords: *Zingiber pellium*, diarylheptanoids, zingiberpyrans A, zingiberpyrans B, anti-inflammation

Introduction

Zingiber pellitum Gapnep. is an endemic species of Vietnam and is found in several regions of this country[1]. It is an herbaceous plant, reaching a height of 0.8-1.2 meters, with densely long hair covering the entire plant and a tuberous rhizome. This species is distinguished by its unique inflorescence type, producing terminal flowers on leafy stems[1]. Like other *Zingiber* species, *Z. pellitum* has a long history of use in traditional medicine to treat ailments such as cold, fever, and cough[2]. A number of compounds have been isolated from different *Zingiber* species, such as diarylheptanoids, gingerols, flavonoids and terpenoids, exhibiting a wide spectrum of bioactivities, such as anti-inflammatory, antioxidant, anti-microbial and anti-cancer[2]. However, previous reports on the chemical composition and biological activity of *Z. pellitum* predominantly focused on essential oil composition[3-5]. The present study, therefore, represents the first to report the isolation of six phenolic compounds (Figure 1), including two new diarylheptanoids, from *Z. pellitum* rhizomes, with their chemical structures determined through spectral analysis. Furthermore, the antioxidant and anti-inflammatory activities of the isolated compounds were also reported in this research.

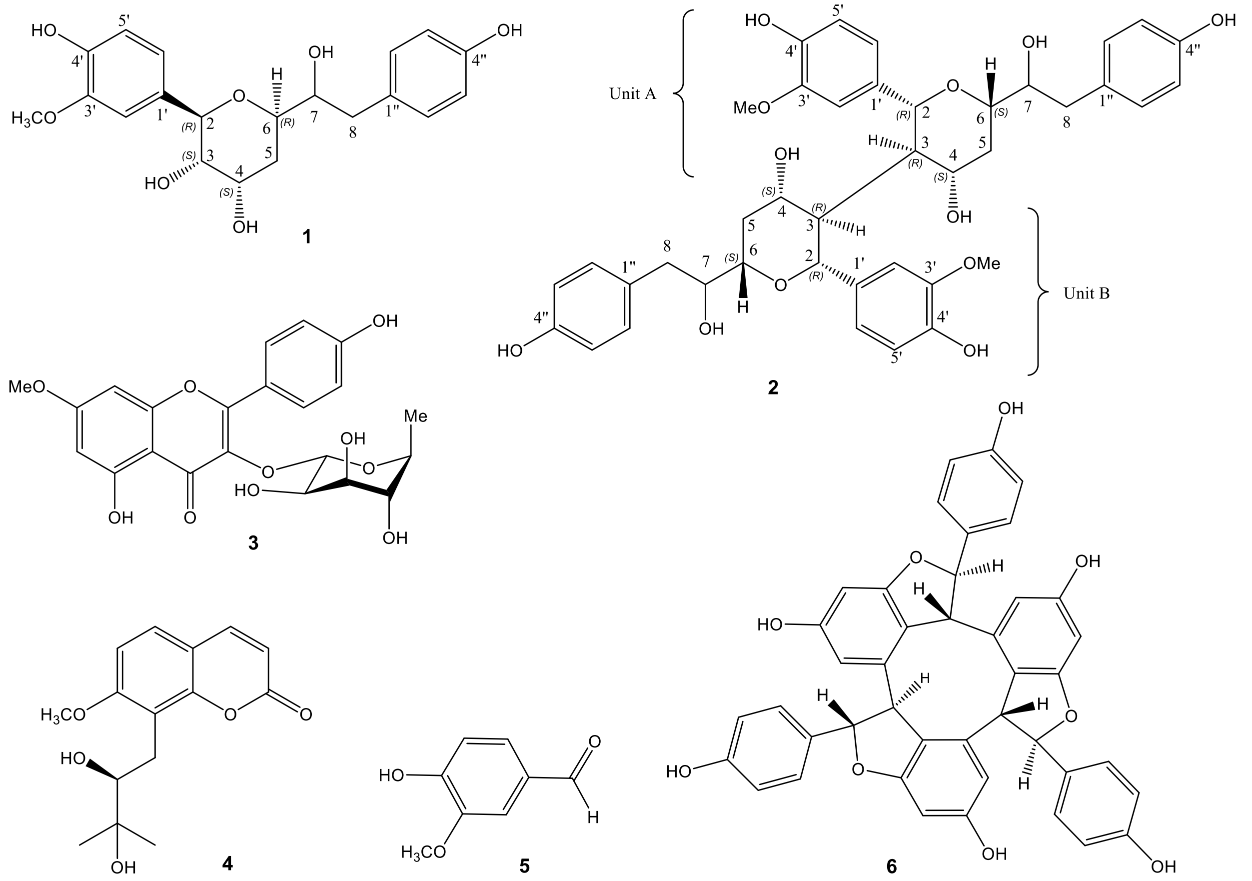


Figure 1. Structures of the compounds isolated from *Z. pellitum* rhizomes.

Results and Discussion

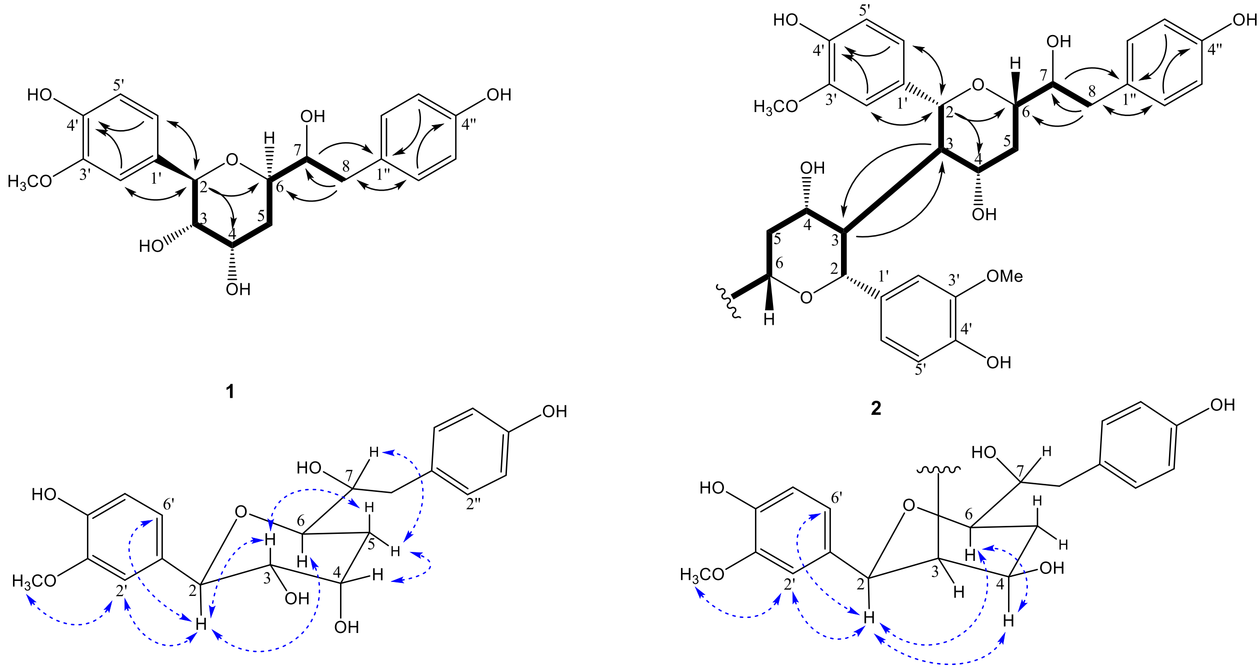
By using various chromatographic techniques, the methanol extract of *Z. pellitum* rhizomes was separated to obtain two new diarylheptanoids **1** and **2**, and four known compounds, rhamnocitrin-3-*O*-rhamnoside (**3**),[6] meranzin hydrate (**4**),[7] vanillin (**5**),[8] and (+)-α-viniferin (**6**).[9]

Compound **1** was obtained as an amorphous pale-yellow powder with the molecular formula $C_{20}H_{24}O_7$, which was confirmed by the sodium adduct at *m/z* 399.1379 $[M + Na]^+$ from the HRESIMS (calcd. for $C_{20}H_{24}NaO_7$, 399.1420). The ^{1}H-NMR spectrum of **1** provided the information of 7 aromatic protons, therein 4 protons at δ_H 7.03 (2H, d, J = 8.4 Hz, H-2′′/6′′) and 6.70 (2H, d, J = 8.4 Hz, H-3′′/5′′) suggesting the presence of an AA′BB′ spin system, and 3 proton signals at δ_H 6.81 (1H, d, *J* = 8.4 Hz, H-5′), 6.94 (1H, d, *J* = 1.8, 7.8 Hz, H-6′), 7.10 (1H, d, *J* = 1.8 Hz, H-2′) were considered to belong to an ABX system (Table 1). The ^{13}C-NMR and HSQC demonstrated the presence of 20 carbon signals, regarding 12 signals belonging to two sets of aromatic systems, five oxymethines at δ_C 69.2, 73.7, 73.9, 75.8, and 79.4, one methoxy group at δ_C 56.5 and two methylene sp^3 carbons δ_C 35.4 and 39.8 (Table 2). In the COSY spectrum, the H-H spin-spin interactions were observed between H-2 (δ_H 4.39) ↔ H-3 (δ_H 3.53) ↔ H-4 (δ_H 4.17) ↔ H-5 (δ_H 1.77, 2.02) ↔ H-6 (δ_H 3.77) ↔ H-7 (δ_H 3.61) ↔ H-8 (δ_H 2.67, 2.83), which allowed for the identification of a continuous bond chain from C-2 to C-8 (Figure 2). The HMBC experiment showed the couplings from H-2 (δ_H 4.39) to C-1′ (δ_C 133.5), C-2′ (δ_C 112.7), C-6′ (δ_C 122.0) and C-4 (δ_C 69.3) and interaction from H-8 (δ_H 2.67, 2.83) to C-1′′ (δ_C 131.1), C-2′′/6′′ (δ_C 131.4) và C-6 (δ_C 73.9) allowing the identification of compound **1** as a diarylheptanoid with two benzene rings at C-2 and C-8 positions. Besides, HMBC couplings from H-2 (δ_H 4.39) to C-6 (δ_C 73.9) confirmed the presence of a cyclic pyran ring (Figure 2). The location of the methoxy group was assigned at C-3′ based on the HMBC correlation from the methoxy signal (δ_H 3.90) to C-3′ (δ_C 147.5).

Table 1. ^{1}H NMR data (mult., *J* in Hz) of **1** and **2**

No	**1** (in CD_3OD)	**2** (in CD_3OD)	**2** (in DMSO-d_6)
2	4.40 (1H, d, 10.2)	5.09 (1H, d, 6.0)	4.91 (1H, d, 4.8)
3	3.55 (1H, dd, 10.2, 3.0)	3.31 (1H, overlapped)	3.09 (1H, s)
4	4.17 (1H, q-like, 3.0)	4.68 (1H, q-like, 3.6)	4.44 (1H, q-like, 3.0)
5	2.05 (1H, ddd, 13.8, 12.0, 2.4)	2.04 (1H, ddd, 13.8, 7.8, 6.0)	1.84 (1H, m)
	1.79 (1H, ddd, 13.8, 3.6, 2.4)	1.82 (1H, m)	1.78 (1H, m)
6	3.78 (1H, dt, 12.0, 3.0)	3.32 (1H, overlapped)	3.20 (1H, s)
7	3.61 (1H, td, 7.2, 3.6)	3.46 (1H, m, H-7),	3.35 (1H, m, H-7),
8	2.85 (1H, dd, 13.2, 6.6)	2.60 (1H, dd, 13.8, 4.)	2.50 (1H, dd, 13.8, 4.2)
	2.69 (1H, dd, 13.2, 7.2)	2.53 (1H, dd, 13.8, 9.0)	2.39 (1H, dd, 13.8, 9.0)
2′	7.10 (1H, d, 1.8)	6.97 (1H, d, 1.8),	6.89 (1H, d, 1.2),
5′	6.81 (1H, d, 8.4)	6.82 (1H, d, 8.4)	6.75 (1H, d, 8.4)
6′	6.94 (1H, dd, 1.8, 7.8)	6.85 (1H, d, 8.4, 1.8)	6.72 (1H, d, 8.4, 1.2)
2′′-6′′	7.03 (2H, d, 8.4)	6.97 (2H, d, 8.4)	6.93 (2H, d, 8.4)
3′′-5′′	6.70 (2H, d, 8.4)	6.68 (2H, d, 8.4)	6.62 (2H, d, 8.4)
OCH_3	3.90 (3H, br s)	3.85 (3H, br s)	3.74 (3H, br s)

This NMR data were almost identical to those of hedycoropyran A and B [11], except for the small difference in the pyran ring. The large coupling constant between H-2 and H-3 ($J_{2,3}$ = 10.2 Hz) indicated that H-2 and H-3 were in di-axial relationship, while H-4 was in an equatorial orientation due to the small coupling constant with H-3 ($J_{3,4}$ = 3.0 Hz).[10] In the NOESY spectrum of **1**, an evident cross-peak between H-2 and H-6 confirmed the axial-orientation of H-6. For the absolute configuration identification, an ECD experiment was recorded. The 2*R*-configuration was determined based on the negative Cotton effect at 225 nm.[11,12] Consequently, (3*S*,4*S*,6*R*) absolute configurations were assigned. However, the configuration of C-6 could not be determined using the present spectroscopic data. Thus, compound **1** was determined to be (2*R*,3*S*,4*S*,6*R*)-6-(-1-hydroxy-2-(4-hydroxyphenyl)ethyl)-2-(4-hydroxy-3-methoxyphenyl)tetrahydro-2*H*-pyran-3,4-diol, for which the name zingiberpyran A is proposed.



1

2

Figure 2. Key COSY (▬), HMBC (→) and NOESY (<----->) correlations of compounds **1** and **2.**

Compound **2** was obtained as an amorphous pale-yellow powder. Its HRESIMS showed a pseudomolecular ion peak at *m/z* 741.2852 $[M + Na]^+$ compatible with the molecular formula $C_{40}H_{46}O_{12}$ of **2**. The NMR data of **2** were similar to those of **1** with two aromatic AA'BB' and ABX systems, five aliphatic methines, two methylenes and one methoxy group. The detailed 2D NMR analysis of **2** (COSY, NOESY, HSQC and HMBC) allowed to assign the ^{1}H and ^{13}C NMR data as in Table 1. The appearance of the upfield-shifted resonance δ_C 58.4 (C-3) instead of δ_C 73.7 in **1** suggested that C-3 was not hydroxylated. Comparing with the NMR data of diarylheptanoids previously reported [13, 14], this chemical shift is compatible with the C–C connection. In combination with the molecular formula $C_{40}H_{46}O_{12}$ as deduced from the HRESIMS experiment, two identical moieties, A and B, were proposed and linked via a C-3(A)▬C-3(B) bridge. This connection was also supported by HMBC analysis which revealed a clear cross-peak between the proton at δ_H 3.31 (H-3) and the "itself" carbon atom at δ_C 58.4 (C-3) (Figure 2 and S19). It is noted that HMBC experiment gives heteronuclear long-range proton–carbon correlations but not direct one-bond correlations. Thus, the coupling from the proton at δ_H 3.31 to the carbon atom at δ_C 58.4 was assigned for the correlation from H-3 (A) to C-3(B) and vice versa.

Table 2. ^{13}C NMR data of **1** and **2**

No	**1** (in CD_3OD)	**2** (in CD_3OD)	**2** (in DMSO-d_6)
2	79.4	81.1	78.1
3	73.7	58.4	56.6
4	69.3	79.8	77.5
5	35.4	36.6	34.8
6	73.9	72.4	70.5
7	75.8	76.2	73.9
8	39.8	40.0	38.4
1′	133.5	135.8	134.7
2′	112.7	111.7	110.7
3′	148.7	149.2	147.4
4′	147.2	147.4	145.6
5′	115.7	116.3	115.2
6′	122.0	120.8	118.7
1′′	131.1	131.4	130.1
2′′-6′′	131.4	131.3	129.9
3′′-5′′	116.0	116.0	114.6
4′′	156.7	156.5	155.1
OCH_3	56.5	56.5	55.6

Similar to **1**, compound **2** exhibited the negative Cotton effect at 230 nm in the ECD spectrum, indicating 2*R*-configuration. For the relative configurations of the pyran ring, the coupling constants and NOESY correlations were analyzed. Because signals of H-3 and H-6 overlapped in the ^{1}H NMR spectrum of **2** recorded in CD_3OD, the NMR data in DMSO-d_6 of **2** is provided as well (see supplemental material). The smaller coupling constant between H-2 and H-3 ($J_{2,3}$ = 6.0 Hz in CD_3OD and 4.8 in DMSO-d_6) indicated that H-2 and H-3 were in an axial-equatorial relationship but not di-axial in case of **1**. The NOE correlations of H-2/H-4, H-2/H-6 and H-4/H-6 confirmed the same orientation of H-2, H-4 and H-6. From these evidences, compound **2** was newly elucidated to be (2*R*,2'*R*,3*R*,3'*R*,4*S*,4'*S*,6*S*,6'*S*)-6,6'-bis(1-hydroxy-2-(4-hydroxyphenyl)ethyl)-2,2'-bis(4-hydroxy-3-methoxyphenyl)octahydro-2*H*,2'*H*-[3,3'-bipyran]-4,4'-diol, for which the name zingiberpyran B is proposed.

All the isolated compounds were evaluated for their anti-inflammatory effect via inhibition of nitric oxide production. In RAW264.7 cells stimulated by lipopolysaccharide (LPS), rhamnocitrin-3-*O*-rhamnoside (**3**) exhibited the strongest effect with the IC_{50} value of 49.6 ± 0.73 µM while zingiberpyran A (**1**) proved less active (IC_{50} 71.0 µM). The other compounds showed any remark inhibition at 100 µM.

Conclusions

Two new diarylheptanoid compounds named zingiberpyrans A and B, and four known compounds, vanillin, (+)-*α*-viniferin, rhamnocitrin-3-*O*-rhamnoside, and meranzin hydrate were isolated for the first time from the rhizomes of *Zingiber pellitum*. Their structures were elucidated by using NMR, HR-ESI-MS and CD spectral data. All isolated compounds were evaluated for the inhibition of NO production in the LPS-stimulated RAW264.7 cells. Compound **1** and **3** showed remarkable inhibitory activity with IC_{50} value of 71.0 and 49.6 µM, respectively.

Experimental Section

General:

Plant material

Zingiber pellium was collected from Binh Chau-Phuoc Buu Nature Reserve, Ba Ria - Vung Tau, Vietnam in January 2023 and was authenticated by one of the authors, Dr. Nguyen Quoc Binh. A voucher specimen (NPH1.2023) is deposited at the herbarium of the Institute of Ecology and Biological Resources.

Chemical and apparatus

Thin layer chromatography (TLC) was conducted on pre-coated silica gel (60 F254, Merck) and detected under ultraviolet light and sprayed with aqueous sulfuric acid 10%, vanillin 10% or ceric sulfate, then heated at 105°C until the spots appear clearly. Column chromatography (CC) was performed in silica gel (230-400 mesh ASTM, Merck), and Diaion HP 20 (Merck). HPLC system was used as Agilent HPLC 1100 series, coupling with detector DAD, and a semi-preparative process was performed on YMC-pack ODS-A 250x10 mm, 5µm, 20 nm column at a flow rate of 3.0 mL/min.

NMR spectra were recorded on a Bruker AvanceNEO 600 MHz and a Bruker 500MHz spectrometer using TMS-tetramethyl silane as an internal standard. HR-MS were collected on the Agilent 6530 Accurate-Mass Q-TOF LC/MS. Optical rotations were recorded on a Jasco P-2000 Digital Polarimeter.

Extraction and isolation

1.3 kg of dried powder of *Zingiber pellium* (ZP) was extracted exhaustively with methanol. Three methanol extractions (1.5L/time) were filtered, combined and concentrated under reduced pressure to obtain a concentrated total extract. This was partitioned with *n*-hexane and methanol to give the corresponding extracts: *n*-hexane extract (15.93 g) and methanol extract (39.13 g).

The methanol extract was loaded on the Diaion HP-20 CC with stepwise elution water, methanol 40%, methanol 100% and acetone 100% in order to collect 4 fractions M0, M40, M100 and A100 respectively. M100 fraction was chromatographed on silica gel CC and eluted with gradient elution of dichloromethane/methanol (10/1 – 1/1, *v/v/*) to collect 8 fractions (from F1.1 to F1.8). Fraction F1.3 was separated by silica gel CC with hexane/ethyl acetate (4/1/, *v/v/*) followed by preparative HPLC (30-100% MeOH in H_2O in 120 min) to afford compound **5** (3.6 mg). Fraction F1.6 was separated on a column with silica gel (stationary phase) and dichloromethane/acetone (5/1, *v/v/*) (mobile phase), to produce 8 fractions, denoted as F2.1 to F2.8. Fraction F2.6 was continually chromatographed on HPLC (40-80% MeOH in H_2O (HPLC grade) in 180 min) to yield compounds **1** (9.3 mg) and **2** (4.1 mg). Compound **6** was separated from fraction F2.8 by preparative HPLC eluting (30-80% MeOH in H_2O (HPLC grade) in 200 min). Compounds **3** (3.0 mg) and **4** (2.7 mg) were isolated from fraction F1.7 by semi-preparative HPLC eluting with gradient solvent from 30 to 70% MeOH in 180 min.

Zingiberpyran A (**1**): amorphous pale yellow powder; $[\alpha]_D^{28} = -19.6$ (*c* 0.10, MeOH); ECD (MeOH) λmax (Δε) 202 (−14.8), 225 (−1.22); HR-ESI-MS *m/z* = 399.1379 $[M + Na]^+$ (calcd. 399.1420 for $C_{20}H_{24}O_7Na$). ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD), see Table 1 and 2.

Zingiberpyran B (**2**): amorphous pale yellow powder; $[\alpha]_D^{28} = -17.4$ (*c* 0.03, MeOH); ECD (MeOH) λmax (Δε) 206 (−5.6), 230 (−4.96); HR-ESI-MS *m/z* 741.2852 $[M + Na]^+$ (calcd. 741.2887 for $C_{40}H_{46}NaO_{12}$). ^{1}H-NMR (600 MHz, CD_3OD and DMSO-*d*$_6$) and ^{13}C-NMR (150 MHz, CD_3OD and DMSO-*d*$_6$), see Table 1 and 2.

Inhibition assay for nitric oxide production

The inhibitory effect of four isolated compounds was determined using a previously reported procedure[15] with dexamethasone used as a positive control (IC_{50} 13.3 µM). All experiments were performed in triplicate.

Acknowledgements

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Supplementary Material

Full experimental detail including NMR, HRESIMS and ECD spectra of compounds **1** and **2** can be found via the "Supplementary information" section of this article's webpage.

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