

A NEW ANHYDRIDE OF *Alpinia officinarum*

C. Y. Chen,^{1*} C. L. Kao,² H. C. Yeh,¹ H. T. Li,^{3*}
M. J. Cheng,⁴ W. J. Li,⁵ and M. D. Wu⁶

One new anhydride, alpinofficinol (1) was isolated from the rhizomes of Alpinia officinarum Hance (Zingiberaceae). The structure was determined by means of HR-ESI-MS and extensive 1D and 2D NMR spectroscopic studies.

Keywords: *Alpinia officinarum*, Zingiberaceae, rhizomes, anhydride.

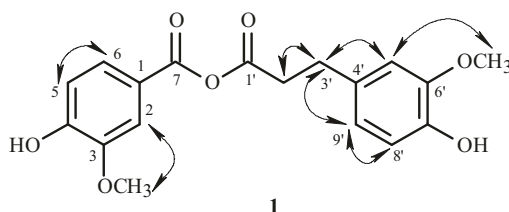
Traditional Chinese medicine consists of a large number of plants with various medicinal and pharmacological importance and hence represents a priceless and invaluable reservoir of new bioactive molecules. *Alpinia officinarum* Hance (Zingiberaceae), also known as lesser galangal, is indigenous to Southeast China and Indochina, and the plains of West Bengal, Assam, and the Eastern Himalayas in India [1]. It is a perennial herb with thick, creeping reddish-brown rhizomes, lineolate acuminate ornamental leaves, and showy white flowers in racemes. It has been used conventionally both in Ayurvedic and Chinese medicine since very early times and in Europe since the Middle Ages [1]. The rhizome has been used in China to relieve stomach ache, treat colds, invigorate the circulatory system, and reduce swelling. The dry root and rhizome have been used for their antioxidant, antidiarrheal, anti-emetic, analgesic, anti-inflammatory, and anticoagulative effects [2]. In the course of screening for biologically and chemically novel agents from Formosan Zingiberaceous plants [3–15], *A. officinarum* was chosen for further phytochemical investigation. In continuation of some studies of chemotaxonomy and biologically active metabolites from this plant, a methanol extraction of the rhizomes of *A. officinarum* afforded ten compounds, including one new anhydride, two phenylalkanoids, two diarylheptanoids, and five benzenoids [16]. In this paper, we report the isolation and structural elucidation of this new propanoic anhydride.

Compound **1**, obtained as yellowish oil, had the molecular formula C₁₈H₁₈O₇, as determined by HR-ESI-MS data *m/z* 369.0955 [M + Na]⁺ (calcd 369.0950) in combination with its ¹H NMR, ¹³C NMR, and DEPT, requiring 10 degrees of unsaturation. The IR spectrum revealed the presence of an OH stretch band (3400 cm⁻¹), and multiple ester carbonyls C=O (1650 cm⁻¹), one of which was by UV spectrum analysis (λ_{max}, 210 (2.75), 255 (3.55), 280 (2.38) nm) in conjugation with a benzene. Eight of the ten degrees of unsaturation inherent in the formula were accounted for by ¹³C NMR as two aromatics and the remaining 2 IHD belong to a typical anhydride signal (δ 169.1 and 176.3). Accordingly, the compound **1** contained an anhydride combined with two benzene rings.

1) School of Medical and Health Sciences, Fooyin University, Ta-Liao, 83102, Kaohsiung, Taiwan, e-mail: xx377@fy.edu.tw; 2) Tzu Hui Institute of Technology, Pingtung County, Taiwan; 3) Department of Medical Laboratory Science and Biotechnology, Fooyin University, Ta-Liao, 83102, Kaohsiung, Taiwan, e-mail: mt085@fy.edu.tw; 4) Department of Life Science, Fu Jen Catholic University, New Taipei City, 24205, Taiwan, e-mail: chengfirdi@gmail.com; 5) Department of Nursing, Fooyin University, Ta-Liao, 83102, Kaohsiung, Taiwan; 6) Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI), Hsinchu 300, Taiwan. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2023, pp. 405–407. Original article submitted March 7, 2022.

TABLE 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR Data of **1** (CD_3OD , δ , ppm, J/Hz)

C atom	δ_{H}	δ_{C}	HMBC
1	–	132.8	–
2	7.51 (d, $J = 2.0$)	112.8	C-1, 6, 7
3	–	147.4	–
4	–	151.0	–
5	6.83 (d, $J = 8.4$)	114.8	C-3, 4, 6
6	7.56 (dd, $J = 8.4, 2.0$)	124.5	C-7, 2, 4
7	–	169.1	–
1'	–	176.3	–
2'	2.53 (t, $J = 7.2$)	36.5	C-1', 3', 4'
3'	2.82 (t, $J = 7.2$)	30.9	C-1', 2', 5', 9'
4'	–	132.8	–
5'	6.69 (d, $J = 2.0$)	111.8	C-3', 4', 6', 7'
6'	–	147.2	–
7'	–	144.4	–
8'	6.72 (d, $J = 8.0$)	115.1	C-6', 9'
9'	6.62 (dd, $J = 8.0, 2.0$)	120.9	C-4', 5'
4-OCH ₃	3.86 (s)	55.9	C-4
6'-OCH ₃	3.81 (s)	56.0	C-6'

Fig. 1. NOESY correlations of **1**.

The ^1H and ^{13}C NMR spectra indicated eight quaternary C atoms, 6 CH, 2 CH_2 , and 2 OMe groups. In the ^1H NMR spectrum, there were typical signals for two OMe groups at δ 3.86 (3H, s, 3-OCH₃), and 3.81 (3H, s, 6'-OCH₃), signals of α -methylene protons of a ketone at δ 2.53 (2H, t, $J = 7.2$ Hz, CH_2 -2'), one β -methylene resonance of a ketone at δ 2.82 (2H, t, $J = 7.2$ Hz, CH_2 -3'), one ABX signal at δ 6.69 (1H, d, $J = 2.0$ Hz), 6.72 (1H, d, $J = 8.0$ Hz), and 6.62 (1H, dd, $J = 8.0, 2.0$ Hz) for H-5', H-8', and H-9' as well as another ABX at δ 7.51 (1H, d, $J = 2.0$ Hz), 6.83 (1H, d, $J = 8.4$ Hz), and 7.56 (1H, dd, $J = 8.4, 2.0$ Hz) for H-2, H-5, and H-6, indicating that **1** was probably a propanoic anhydride possessing 4-hydroxy-3-methoxybenzoic and 3-(4-hydroxy-3-methoxyphenyl) groups. The carbons of the propanoic anhydride were assigned, from ^{13}C NMR and DEPT experiments; there were resonances for anhydride functions [δ 169.1 (C-7) and 176.3 (ester C=O groups)], two methoxyl groups [δ_{C} 55.9, 56.0], and two aromatic C atoms. The above data of **1** also pointed to a propanoic anhydride moiety.

The above observation accompanied by the ^1H - ^1H COSY (Table 1), and HMBC (Table 1) spectrum of **1** established the presence of the partial three substitutes: fragments, **1a** (4-hydroxy-3-methoxybenzoic group), **1b** (3-(4-hydroxy-3-methoxyphenyl) group), and **1c** ($-\text{OCOCH}_2\text{CH}_2-$, propanoic anhydride group), for anhydride skeleton of **1**. The entire skeleton of **1** was constructed with the aid of HMBC spectrum (Table 1).

In the HMBC and NOESY spectra, the significant correlations between δ_{H} 7.51 (H-2)/ δ_{H} 7.56 (H-6) and δ_{C} 169.1 (C-7), δ_{H} 6.69 (H-5')/ δ_{H} 6.62 (H-9') and δ_{C} 30.9 (C-3'), suggested that the substitutes **1a** and **1b** were connected to C-1 and C-4' of the propanoic anhydride moiety respectively. The other key HMBC correlations of **1** demonstrated selected cross-peaks as shown in Table 1. The assignments were further verified by significant correlations of H-3' (δ 2.82)/H-5' (δ 6.69) and H-9' (δ 6.62) in the NOESY experiments (Fig. 1) and further supported the positions of each substituent (**1a** & **1b**) on the anhydride moiety.

From the above data, compound **1** was characterized as 4-hydroxy-3-methoxybenzoic 3-(4-hydroxy-3-methoxyphenyl)propanoic anhydride, named alpinofficinol, and its structure was illustrated as **1**, which was further confirmed by COSY, NOESY (Fig. 1), HSQC, and HMBC (Table 1) experiments.

EXPERIMENTAL

General. UV spectra were obtained in CH₃CN, IR spectra were measured on a Hitachi 260-30 spectrophotometer. ¹H NMR (400 MHz), ¹³C NMR (100 MHz), HSQC, HMBC, COSY, NOESY, and DEPT spectra were obtained on a Varian (Unity Plus) NMR spectrometer. Low-resolution ESI-MS spectra were obtained on an API 3000 (Applied Biosystems) and high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer. Silica gel 60 (Merck, 70–230 mesh, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254), 0.20 mm and 0.50 mm, were used for analytical TLC and preparative TLC respectively, visualized with 50% H₂SO₄.

Plant Material. The rhizomes of *A. officinarum* were collected from Taoyuan County, Taiwan, in June 2017. Plant material was identified by Dr. Fu-Yuan Lu (Department of Forestry and Natural Resources College of Agriculture, National Chiayi University). A voucher specimen was deposited at the Department of Medical Technology, School of Medical and Health Sciences, Fooyin University, Kaohsiung, Taiwan.

Extraction and Isolation. The rhizomes (3.24 kg) of *A. officinarum* were extracted repeatedly with MeOH (5 L × 3) at room temperature for 24–48 h. The MeOH extract was dried and evaporated to leave a viscous residue (112.4 g). The residue was placed on a silica gel column (3.5 kg, 70–230 mesh) and eluted with CH₂Cl₂ gradually enriched with MeOH to afford ten fractions. Fraction 2 (25.8 g) was subjected to silica gel chromatography (1.4 kg, 70–230 mesh), by eluting with *n*-hexane–acetone (80:1), enriched gradually with acetone, to furnish four fractions (2-1–2-4). Fraction 2-4 (7.5 g) was further purified on a silica gel column using *n*-hexane–acetone (60:1) to obtain alpinofficinol (**1**) (11 mg).

Alpinofficinol (1), colorless oil. UV (MeCN, λ_{max}, nm) (log ε): 210 (2.75), 255 (3.55), 280 (2.38). IR (ν_{max}, cm⁻¹): 3400 (OH), 1650 (C=O). ESI-MS *m/z* 369 [M + Na]⁺; HR-ESI-MS *m/z* 369.0955 [M + Na]⁺ (calcd for C₁₈H₁₈O₇Na, 369.0950). ¹H and ¹³C NMR data, see Table 1.

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