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Cytotoxic Substances Produced by *Erioscyphella abnormis* Strain FC-2579

Yuta Hama[1], Daigo Wakana[1], Yuji Doi[1], Hisashi Takeda[1], Nobutomo Ikarashi[1], Satoshi Kitaoka[1,2], Fumiaki Sato[1,3], Hiyori Itagaki[4], Tsuyoshi Hosoya[4], Tomoo Hosoe[1*]

[1]School of Pharmacy and Pharmaceutical Sciences, Hoshi University, Tokyo, Japan
[2]Faculty of Pharmacy and Pharmaceutical Sciences, School of Pharmacy, Josai University, Saitama, Japan
[3]Faculty of Pharmacy, Juntendo University, Chiba, Japan
[4]Department of Botany, National Museum of Nature and Science, Ibaraki, Japan
Email: takeda@hoshi.ac.jp, ikarashi@hoshi.ac.jp, skitaoka@josai.ac.jp, fsato@juntendo.ac.jp, hiyori_itagaki@river-museum.jp, hosoya@kahaku.go.jp, *hosoe@hoshi.ac.jp

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Abstract

A chemotaxonomic study was conducted using secondary metabolites produced by *Erioscyphella abnormis* FC-2579, a fungus belonging to the family Lachnaceae of the order Helotiales, and chemical constituents of *Lachnum*, a closely related species of *Erioscyphella*. Although several Helotiales fungi are known to produce bioactive secondary metabolites, the chemical constituents of the genus Erioscyphella have not previously been investigated. Initial cytotoxicity screening of *E. abnormis* FC-2579 extract against HepG2, HL60, and PANC-1 cancer cell lines revealed significant activity, leading to detailed chemical investigation. Through a meticulous isolation process using various chromatographic techniques, two novel lachnochromonin derivatives, namely (+)-lachnochromonin D (**1**) and (+)-lachnochromonin G (**2**), were isolated along with the known compounds caprylic acid (**3**), lachnochromonin C (**4**), lignicol (**5**), 6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**6**), and 6-demethylkigerin (**7**). Structural elucidation was performed using HR-ESI-MS and comprehensive 1D and 2D NMR analysis. The absolute configurations of the novel compounds were estimated by comparative analysis of the optical rotations of structurally similar known compounds. Subsequent cytotoxicity assays demonstrated that **1**, **2**, and **4** exhibited significant, concentration-dependent cytotoxicity against HepG2 and HL60 cells, while **7** showed only mild activity against HL60 cells. None of the tested compounds exhibited cytotoxicity against PANC-1 cells under the experimental conditions. Furthermore, comparative HPLC profiling of six *Erioscyphella* and six *Lachnum* strains revealed that caprylic acid (**3**) was consistently detected only in *Erioscyphella* extracts, suggesting its potential as a chemotaxonomic marker.

Keywords

Erioscyphella abnormis, Lachnaceae, Cytotoxicity, Lachnochromonin, Caprylic Acid, Chemotaxonomy

1. Introduction

The order Helotiales is one of the most diverse groups of Ascomycetes, with approximately 4000 species currently discovered worldwide [1]-[4].

Fungi belonging to Helotiales are well recognized as producers of structurally diverse and biologically active secondary metabolites. Examples include F-36316 A, which exhibits angiogenesis-promoting activity [5], lachnellins A-D with cytotoxic activity [6], and quinone derivatives that modulate drug efflux pumps [7].

In our previous study, (+)-phaeosphaerin A, (+)-phaeosphaerin B, and (*S*)-6-demethylkigelin were isolated from *Phialocephala scopiformis* FC-1873 which belong to Helotiales, and cytotoxicity of these compounds were reported [8].

In this study, we focused on the genus *Erioscyphella* (Helotiales), which has not reported any chemical constituents in our knowledge, but which is expected to produce physiologically active substances as mentioned above. Various biologically active substances have been reported from *Lachnum* spp. [9]-[13], a species closely related to *Erioscyphella* spp.

In contrast, no chemical investigation of the genus *Erioscyphella* has been reported to date, despite its close phylogenetic relationship to metabolite-producing genera such as *Lachnum*.

The classification of the genus *Erioscyphella* and genus *Lachnum* has been confused, but the classification of both genera has been proposed based on molecular biological analysis and redefined morphological study [14]. To gain further information on this classification, we investigated the chemical components of *Erioscyphella* species to obtain chemotaxonomic information and searched for useful substances.

2. Materials and Methods

2.1. Experimental Instruments

For column chromatography, Silicagel60 (KANTO CHEMICAL CO., INC. Tokyo, Japan) was used. HPLC for isolation, HPLC pump LC-20AT (Shimadzu, Kyoto, Japan), UV detector SPD-20AV (Shimadzu) and HPLC column Inertsustain C18 (5 μm, 10 × 250 mm), and chromatographic condition as below; flow rate: 2 mL/min, detected wavelength; 210 nm. Analytical HPLC was performed using a Shimadzu LC-20AT system equipped with an ODS column (Mightysil RP-18 GP, 5 μm, 3.0 × 250 mm) and an SPD-M20A Diode array detector. NMR spectra were measured by ECAII 600 spectrometer (JEOL, Tokyo, Japan). The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. Optical rotation measured by DIP-1000 polarimeter (JASCO) and UV spectra measured by

Ultrospec 2100 pro spectrophotometer. ESI-MS were measured at negative mode on T-100LP mass spectrometer (JASCO).

A microplate reader (AS ONE MPR-A100) was used to measure absorbance in the MTT assays.

2.2. Fungal Material

Six strains of *Erioscyphella* (including *E. abnormis* strain FC-2579) and six strains of *Lachnum* used in this study were all provided by Dr. Tsuyoshi Hosoya (Department of Botany, National Museum of Nature and Science, Japan). Detailed collection information (collection site, year, month, and substrate) for these strains is summarized in Table 1.

Table 1. Fungal information used in this study.

strain	location	date	substrate
Erioscyphella sclerotii FC-1966-1	Hahajima, Ogasawara islands, Tokyo	24, 6, 2009	decayed wood
Erioscyphella sclerotii FC-1966-2	Hahajima, Ogasawara islands, Tokyo	24, 6, 2009	decayed wood
Erioscyphella abnormis FC-2579	Iryuda, Odawara City, Kanagawa	14, 5, 2010	decayed wood
Erioscyphella abnormis FC-2147	Yamakita-machi, Ashigarakami-gun, Kanagawa	2, 7, 2005	decayed wood
Erioscyphella abnormis FC-5693	Niiharu-cho, Yokohama, Kanagawa	1, 4, 2015	decayed wood
Erioscyphella abnormis FC-6629	Oyama-cho, Sunto-gun, Shizuoka	26, 6, 2017	decayed wood
Lachnum palmae FC-1846	Kasari-cyo, Amami City, Kagoshima	24, 2, 2009	leaf of *Livistona chinensis*
Lachnum palmae FC-1856	Sumiyo-cyo, Amami City, Kagoshima	25, 2, 2009	leaf of *Livistona chinensis*
Lachnum palmae FC-1935	North Iwo Jima, Ogasawara islands, Tokyo	17, 6, 2009	leaf of *Livistona chinensis*
Lachnum palmae FC-1938	North Iwo Jima, Ogasawara islands, Tokyo	18, 6, 2009	leaf of *Livistona chinensis*
Lachnum palmae FC-1957	Chichijima Island, Ogasawara islands, Tokyo	27, 6, 2009	leaf of *Livistona chinensis*
Lachnum palmae FC-2712	Iriomote Island, Okinawa	13, 6, 2011	leaf of *Livistona chinensis*

2.3. Cultivation and Extraction

Each strain was first grown on potato dextrose agar (PDA; Nissui Pharmaceutical Co.) and then inoculated into a sterile rice medium (100 g of autoclaved white rice and 100 mL of sterile water in a 500 mL Roux bottle). The cultures were incubated statically at 25˚C for 3 weeks. After incubation, methanol was added to the solid rice cultures and left overnight. The mixture was then filtered to obtain a crude extract. For strain FC-2579, approximately 8.24 g of crude methanol extract was obtained from about 8 L of total culture volume. All fungal cultures were performed using a SANYO MIR-153 incubator.

2.4. Isolation and Purification of FC-2579 Components

The crude methanol extract of FC-2579 was suspended in water and partitioned twice with an equal volume of ethyl acetate (EtOAc). The residual aqueous layer was further extracted with *n*-butanol. Based on the results of the cytotoxicity assay

(described below), the EtOAc-soluble fraction of *E. abnormis* FC-2579 showed the most potent activity; therefore, this fraction (1.64 g) was used for subsequent purification. The EtOAc extract (1.64 g) was subjected to silica gel column chromatography (silica gel 60; column dimensions 5 × 80 cm), eluted stepwise with hexane/acetone mixtures (starting at 10:1, then stepwise changing to 5:1, 3:1, 1:1, followed by 100% acetone and finally 100% methanol). Eluates were concentrated and combined into 10 fractions (Fr. A-J). Fraction F was purified by ODS-HPLC (eluent: 70% acetonitrile) to obtain (+)-lachnochromonin D (**1**: 4.4 mg) and caprylic acid (**3**: 3.9 mg) and three fractions (Fr. F-1 to F-3). Fr. F-2 was further purified by ODS-HPLC (eluent: 70% methanol) to get (+)-lachnochromonin G (**2**: 4.2 mg). Fr. I was separated by ODS-HPLC (eluent: 70% acetonitrile) to obtain lachnochromonin C (**4**: 5.6 mg) and four fractions (Fr. I-1 to I-4). Fr. I-3 was purified by ODS-HPLC (eluent: 70% methanol) to obtain lignicol (**5**: 3.4 mg), 6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**6**: 3.6 mg) and 6-demethylkigelin (**7**: 4.5 mg).

2.5. MTT Cytotoxicity Assay for 1 - 7

The cytotoxic activity of the isolated was evaluated using an MTT assay. Three human cancer cell lines were used: liver hepatocellular carcinoma (HepG2), promyelocytic leukemia (HL-60), and pancreatic carcinoma (PANC-1). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) or RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37˚C in a humidified atmosphere with 5% CO_2. Cells were seeded in 96-well plates at 5.0×10^3 cells per well and incubated for 48 hours. The test compounds were dissolved in DMSO and added to the wells at a final concentration range of 1.56 to 100 μM, with the final DMSO concentration maintained below 0.5% (v/v) to ensure no vehicle-induced toxicity. Incubation was continued for 24 hours. After treatment, 10 μL of MTT reagent (Nakalai Tesque, Japan) was added to each well and the plates were incubated for 3 hours at 37˚C. The formazan produced by viable cells was dissolved (with the reagent's supplied solvent), and the absorbance at 570 nm was measured using the microplate reader. Cell viability was calculated as the absorbance of the treated sample relative to that of the untreated control. The average and standard deviation (SD) of four replicate wells were calculated for each treatment.

2.6. HPLC Analysis for Chemical Profile Comparison

Six strains of *Erioscyphella* and six strains of *Lachnum* were cultured on the solid-state rice medium under the same conditions (25˚C, 21 days) and extracted with methanol. Each resulting crude methanol extract was evaporated to dryness, and 1 mg of the residue was dissolved in a 1:1 (v/v) mixture of water and acetonitrile. The solution was centrifuged, and the supernatant was used as the sample for HPLC analysis. Analytical HPLC was performed as described above on the ODS column; the mobile phase was water (solvent A) and acetonitrile (solvent B) with

a linear gradient from 20% B to 90% B over 40 minutes, followed by an isocratic hold at 90% B until 50 minutes. The flow rate was 0.5 mL/min, the column temperature was 40˚C, and UV detection was at 210 nm. Blank rice-medium extracts and solvent controls were processed and analyzed under the same conditions; caprylic acid (**3**) was consistently absent in these controls, confirming its fungal origin.

The HPLC chromatograms of the extracts were compared between the two genera to identify peaks common to or characteristic of each genus.

2.7. Statistical Analysis

Statistical analysis for significant differences compared to the control was performed using Dunnett's multiple comparison test (R software, multcomp package).

3. Results

The crude extract of *E. abnormis* strain FC-2579 was purified by silicagel column chromatography and ODS-HPLC, and compounds **1** and **2** were new isolates obtained in this study, whereas **3** - **7** were identified as known compounds (Figure 1). The structures of these compounds were elucidated by various spectroscopic methods.

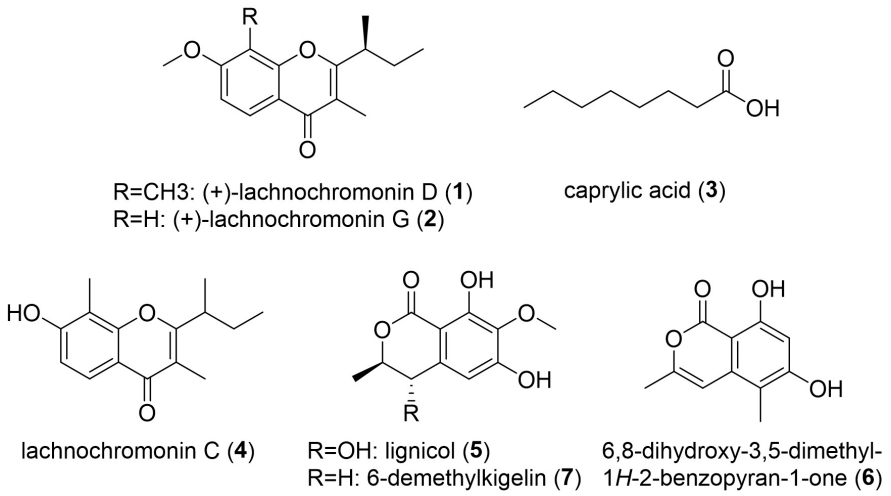

Figure 1. Structure isolated from *E. abnormis* strain FC-2579 in this study.

3.1. Structural Elucidation of 1 and 2

(+)-Lachnochromonin D (1)

Compound **1** was isolated as a colorless oil. HR-ESI-MS analysis determined its molecular formula to be $C_{16}H_{20}O_3$ (m/z 261.1514 $[M + H]^+$, calcd. 261.1506 for $C_{16}H_{21}O_3$). The UV spectrum showed maximum absorption at 231, 246, 254, and 298 nm, which was similar to other lachnochromonins reported from *Lachnum* species [13]. The ^{1}H-NMR spectrum revealed two aromatic proton signals characteristic of a 1,2,3,4-substituted benzene ring (δ_H 8.04 (d, J = 8.9 Hz), 6.92 (d, J = 8.9 Hz)), five methyl group signals including one oxygenated proton (δ_H 3.91 (s),

2.26 (s), 2.04 (s), 1.29 (d, J = 6.8 Hz), 0.89 (t, J = 7.2 Hz)), and three other signals (δ_H 3.00 (m), 1.82 (m), 1.69 (m)). The ^{13}C-NMR spectrum showed nine sp^2 carbons including one carbonyl carbon (δ_C 178.4), five methyl carbons including one oxygenated carbon (δ_C 56.0, 18.0, 11.9, 9.4, 7.8), one methylene carbon (δ_C 27.8), and one methine carbon (δ_C 37.6). These NMR spectral data were in complete agreement with lachnochromonin D, which has been reported to be isolated from *Lachnum abnorme* [13].

The optical rotation value of the known lachnochromonin D was reported to be −33.6˚ (c 0.5, $CHCl_3$) [10], whereas the optical rotation of **1** isolated in this study was +37.4˚ (c 0.5, $CHCl_3$). This suggests that **1** is an enantiomer of known lachnochromonin D.

(+)-Lachnochromonin G (2)

Compound **2** was isolated as a yellow amorphous powder. HR-ESI-MS analysis determined its molecular formula to be $C_{15}H_{18}O_3$ (m/z 247.1326 $[M + H]^+$, calcd. 247.1328 for $C_{15}H_{19}O_3$). The ^{1}H-NMR spectrum revealed three aromatic proton signals characteristic of a 1,2,4-substituted benzene ring (δ_H 6.76 (d, J = 2.1 Hz), 6.92 (dd, J = 2.1, 8.9 Hz), 8.10 (d, J = 8.9 Hz)), four methyl group signals including one oxygenated methyl group (δ_H 3.91 (s), 2.06 (s), 1.29 (d, J = 6.9 Hz), 0.91 (t, J = 7.6 Hz)), and three other signals (δ_H 2.99 (m), 1.80 (m), 1.63 (m)). The ^{13}C-NMR spectrum showed nine sp^2 carbons including one carbonyl carbon (δ_C 177.8), four methyl carbons including one oxygenated carbon (δ_C 55.7, 17.9, 12.0, 9.4), one methylene carbon (δ_C 27.5), and one methine carbon (δ_C 37.4). These data suggested that **2** possessed a chromone skeleton with a *sec*-butyl group, similar to the known lachnochromonin D [13]. Detailed comparison with lachnochromonin D suggested that **2** was a structure lacking the methyl group at C-14 of lachnochromonin D. Analysis of 2D NMR spectra further supported this structural assignment (**Figure 2**). ^{1}H-^{1}H COSY correlations between H-5 (δ_H 8.10) and H-6 (δ_H 6.92), and HMBC correlations from H-5 to C-7 and C-8a, from H-6 to C-8, and from H-8 to C-4a and C-6, confirmed the presence of a 1,2,4-substituted benzene ring. Furthermore, correlations from H-5 to C-4 and HMBC correlations from H-13 to C-2, C-3, and C-4 revealed that the 1,2,4-substituted benzene ring formed a chromone skeleton with a methyl group at C-3. ^{1}H-^{1}H COSY correlations between H-11 and H-10 and H-9 and H-12 indicated the sec-butyl group. HMBC correlations from OCH_3-7 (δ_H 3.91) to C-7 (δ_C 163.5) and H-12 to C-2 suggested the presence of a methoxy group at C-7 and sec-butyl group at C-2. Based on these results, the chemical structure of **2** was confirmed, and given its optical rotation of +42.5˚ (c 0.2, methanol), it was named (+)-lachnochromonin G.

The absolute configurations of the *sec*-butyl group in **1** and **2** were tentatively assigned as *S* based on comparative analysis of the optical rotations of structurally similar known compounds. However, as absolute configuration inference from optical rotation alone has inherent limitations, further studies using orthogonal approaches such as Electronic Circular Dichroism (ECD) spectroscopy or total synthesis are required for definitive confirmation.

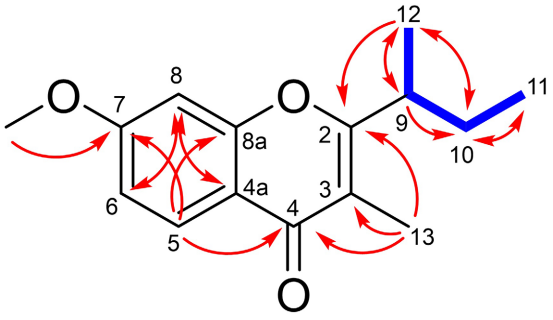

Figure 2. Analyses of ^{1}H-^{1}H COSY and HMBC correlations of **2**.

Five known Compounds (**3** - **7**) were identified by comparison with standard material or reported data; caprylic acid (**3**), lachnochromonin C (**4**) [15], lignicol (**5**) [16], 6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**6**) [17], and 6-demethylkigelin (**7**) [18]. Caprylic acid (**3**) is a medium-chain fatty acid that has been reported to exhibit growth-inhibitory effects on HCT-116 and A-431 cell lines [19], but its report as a secondary metabolite of Helotiales fungi is new to this study. Lachnochromonin C (**4**) was originally isolated from *Crocicreas virgineum* (formerly classified as *Lachnum virgineum*) and is known to be cytotoxic toward HepG2 cells [15]. Lignicol (**5**) and 6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**6**) are isocoumarin derivatives that have been previously isolated from fungi of the order Helotiales (e.g., *Mollisia* sp.); notably, lignicol was reported as a novel metabolite from a white-rot fungus (*Scytalidium* sp.) [16] [17]. Lignicol (**5**) has been reported to show cytotoxicity against HL60 cells, and **6** has a Japanese patent as a synthetic compound with bronchial smooth muscle relaxant activity [8]. 6-Demethylkigelin (**7**) was originally isolated from the ascomycete *Aspergillus terreus* and has been reported to act as a plant root growth promoter [18]. These findings demonstrate that strain FC-2579 produces a diverse array of secondary metabolites, including new lachnochromonin analogues. The structures of **1** - **7** are presented in **Figure 1**.

3.2. Cytotoxicity Evaluation of Isolated Compounds

Among the isolated compounds, **1**, **2**, **4**, **6**, and **7**, for which cytotoxicity had not been previously investigated, were evaluated for cytotoxicity using the MTT assay against HepG2, HL60, and PANC-1 cell lines, which showed cytotoxicity to the rice culture extract of *E. abnormis* FC-2579. As a result, the following activities were confirmed (**Figure 3** and **Table 2**).

Compound **1** reduced cell viability in a concentration-dependent manner in HepG2 and HL60 cells. At 100 μM, HepG2 cell viability decreased to less than 5%. Compound **2** also showed concentration-dependent cytotoxicity against HepG2 and HL60 cells. Compound **4** (Lachnochromonin C) exhibited similar activity. Compound **6** showed no significant cytotoxicity in any tested cell line, even at 100 μM, whereas compound **7** showed mild activity against HL60 cells. Importantly, none of the isolated compounds reduced cell viability in PANC-1 cells.

These findings indicate selective cytotoxic activity toward specific cancer cell types rather than broad-spectrum cytotoxicity.

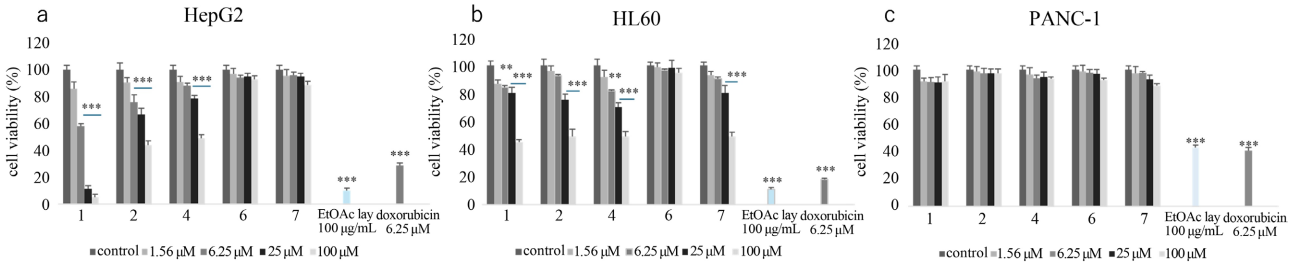

Figure 3. Effects of compounds **1**, **2**, **4**, **6**, **7** on the viability of cancer cells as assessed by MTT assay. Doxorubicin (6.25 μM) was employed as the positive (cell death-inducing) control. Data are shown as means ± SD (n = 3). Comparisons to the (negative) control (no added compound) were performed by Dunnett's multiple comparisons test. Conditions without an asterisk exhibited no significant difference. **p < 0.01, ***p < 0.001. a: HepG2, b: HL-60, c: PANC-1.

Table 2. IC_{50} values (μM) of compounds isolated from *E. abnormis* strain FC-2579.

Compound	HepG2	HL-60	PANC-1
1	12.5 (10.2 - 14.8)	88.4 (75.1 - 102.3)	>100
2	94.2 (82.6 - 105.8)	98.1 (85.4 - 110.8)	>100
4	99.1 (88.3 - 112.5)	96.5 (84.2 - 108.8)	>100
6	>100	>100	>100
7	>100	97.4 (86.5 - 109.2)	>100

Data are expressed as IC_{50} values (μM) with 95% confidence intervals in parentheses. Values > 100 indicate no significant growth inhibition observed at the highest concentration tested.

3.3. Investigation of Classification of *Erioscyphella* and *Lachnum* Genera by Chemical Components

To address the taxonomic challenges of *Erioscyphella* and *Lachnum* genera, which are difficult to classify morphologically and molecular phylogenetically, a chemotaxonomic approach focusing on differences in produced components was applied. In this study, HPLC profiles of rice culture extracts from six *Erioscyphella* strains and six *Lachnum* strains, provided by the National Museum of Nature and Science, were compared.

After culturing each fungal strain on solid state rice medium, methanol extraction was performed, and the obtained methanol extracts were subjected to comparative HPLC analysis (**Figure 4**). First, methanol extracts from six *Erioscyphella* strains were analyzed by HPLC, and comparison of HPLC chromatograms of each strain revealed that only the peak of caprylic acid (**3**), isolated from FC-2579, was consistently observed in the other five strains as well. This suggests that caprylic acid is a common metabolite produced by *Erioscyphella* species. Next, methanol extracts from six *Lachnum* strains were analyzed by HPLC, and comparison of the HPLC chromatograms of each strain revealed no common peaks among the six *Lachnum* strains, with diverse HPLC profiles observed for each strain. Further-

more, and more importantly, the peak of caprylic acid (**3**), which was consistently observed in *Erioscyphella* strains, was not observed in any of the *Lachnum* strain extracts. These results clearly demonstrated that caprylic acid (**3**) was specifically observed only in the culture extracts of *Erioscyphella* species and was completely absent in *Lachnum* species.

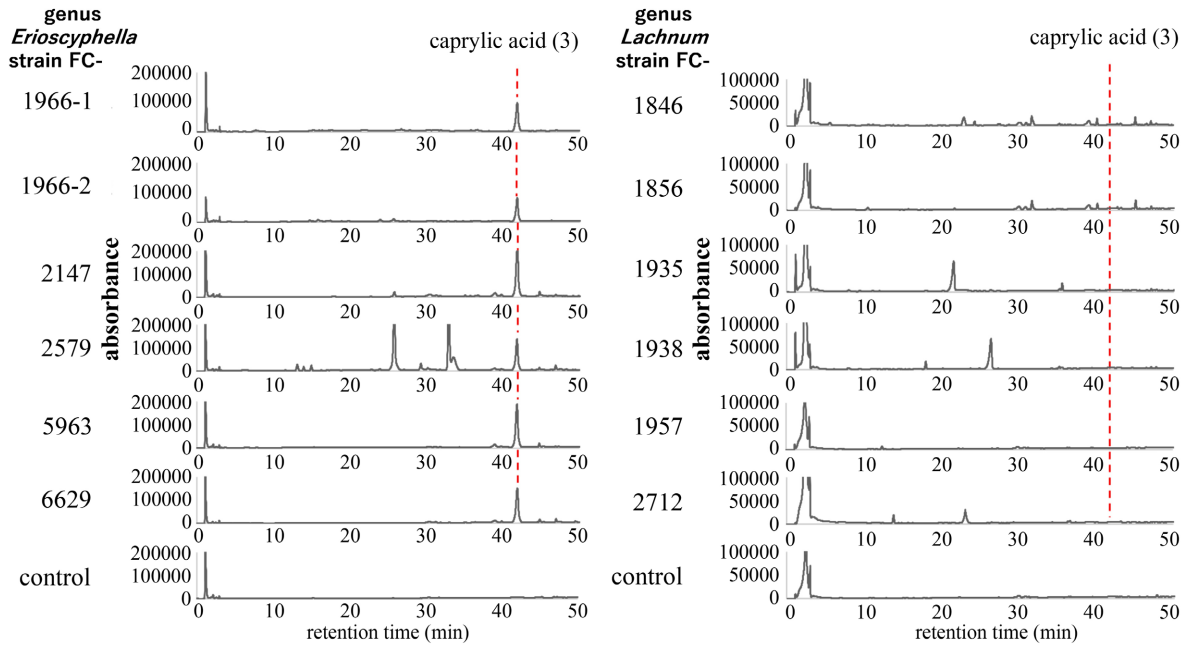

Figure 4. HPLC profile of *Erioscyphella* and *Lachnum* cultured extract. These fungi were cultured on solid-state rice medium and extracted with methanol.

This chemical difference strongly suggests the potential contribution of chemical components to the classification of *Erioscyphella* and *Lachnum* genera. This finding provides a new and complementary perspective for the classification of these two genera, which have been difficult to distinguish based solely on morphological features or molecular phylogenetic markers.

4. Discussion

In this study, two new lachnochromonin derivatives, namely (+)-lachnochromonin D (**1**) and (+)-lachnochromonin G (**2**), were isolated from *E. abnormis* FC-2579.

Compound **1** showed NMR data identical to those of previously reported lachnochromonin D; however, its optical rotation value (+37.4˚, c 0.5, $CHCl_3$) was opposite in sign to that of the reported compound (−33.6˚, c 0.5, $CHCl_3$) [13], indicating that **1** is its enantiomer. Compound **2** represents a new structural analogue lacking the C-14 methyl group of lachnochromonin D. This expands the structural diversity of lachnochromonins within the family Lachnaceae.

To date, six types of lachnochromonins have been discovered; lachnochromonin A, B, and C (all with positive optical rotation) from *L. virgineum*

SANK10207, and lachnochromonin D, E, and F (all with negative optical rotation) from *Lachnum abnorme* BCRC09F0006. Lachnochromonin C, D, and G isolated from *Erioscyphella* species in this study all showed positive optical rotation. These observations indicate that the optical rotation patterns of lachnochromonins differ among producing strains. However, further studies involving additional isolates and absolute stereochemical determination are necessary to clarify whether this tendency is genus-specific.

Selectivity of Cytotoxicity and the Role of Efflux Transporters

Compounds **1**, **2**, and **4** exhibited significant cytotoxicity against HepG2 and HL60 cell lines but showed no activity against PANC-1 cells. Additionally, compound **7** showed mild activity against HL60 cells. These results indicate cell-type-dependent sensitivity rather than broad-spectrum cytotoxicity. The mechanisms underlying this selectivity remain to be elucidated and warrant further investigation.

The observed resistance of PANC-1 cells may be related to differences in intracellular accumulation of the compounds. Chromone and isocoumarin derivatives have been reported to interact with efflux transporters such as P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) [20]. In addition, differences in the expression levels of these transporters among cancer cell lines have been documented [21].

PANC-1 cells are known to express relatively high levels of efflux transporters such as BCRP, which may contribute to the reduced intracellular accumulation of these compounds and the resulting lack of cytotoxicity in this cell line. Alternatively, the observed selectivity may also arise from differences in cellular uptake efficiency or metabolic inactivation rates in different cancer cells. However, these possibilities require further experimental validation.

Role of Caprylic Acid in Chemotaxonomy

Another significant achievement of this study is the suggestion that the production of caprylic acid (**3**) could serve as a potential marker to distinguish between *Erioscyphella* and *Lachnum* species in chemotaxonomic studies.

HPLC analysis revealed that caprylic acid (**3**) was consistently detected in the culture extracts of all six *Erioscyphella* strains, whereas it was completely absent in the extracts of all six *Lachnum* strains. This reproducible chemical difference suggests that caprylic acid (**3**) may serve as a useful chemotaxonomic marker at the genus level under the examined culture conditions. While caprylic acid (**3**) serves as a reproducible marker under our current culture conditions (25˚C, 21 days, rice medium), its robustness as a chemotaxonomic tool may vary depending on the substrate or cultivation parameters, which warrants further validation across diverse growth environments.

Currently, biological classification is predominantly based on molecular biological methods, and the classification of *Erioscyphella* and *Lachnum* species is also being investigated using DNA sequence data from the ITS, LSU, mtSSU, and RPB2 (RNA polymerase II gene) regions of ribosomal DNA [14].

Integration of chemotaxonomic markers with molecular phylogenetic data may provide complementary information for resolving taxonomic relationships within Lachnaceae.

5. Conclusions

This study represents the first chemical investigation of the Japanese isolate *Erioscyphella abnormis* FC-2579 and a comparative chemotaxonomic analysis of *Erioscyphella* and *Lachnum* species.

Chromatographic separation of the rice culture extract of *E. abnormis* FC-2579 yielded two new lachnochromonins, namely (+)-lachnochromonin D (**1**) and (+)-lachnochromonin G (**2**), together with five known compounds: caprylic acid (**3**), lachnochromonin C (**4**), lignicol (**5**), 6,8-dihydroxy-3,5-dimethyl-1*H*-2-benzopyran-1-one (**6**), and 6-demethylkigelin (**7**).

Lachnochromonin C, D, and G isolated from *Erioscyphella* species in this study all showed positive optical rotation. The absolute stereochemistry of the *sec*-butyl group in **1** and **2** was tentatively assigned as *S* based on comparison with reported optical rotation data of structurally related compounds.

Compounds, **1**, **2**, **4**, **6**, and **7** were evaluated for cytotoxicity against cancer cells.

In a MTT assay using HepG2, HL60, and PANC-1 cells, compounds **1**, **2**, and **4** reduced cell viability in HepG2 cells, and **1**, **2**, **4**, and **7** reduced viabilities in HL60 cells. However, none of the compounds reduced cell viability in PANC-1 cells. These findings indicate selective cytotoxic activity depending on the cancer cell type, although the underlying mechanisms remain to be clarified.

The classification of *Erioscyphella* and *Lachnum* species was further examined based on their chemical components. Comparative HPLC analysis revealed that caprylic acid (**3**) was consistently detected only in culture extracts of *Erioscyphella* species and not in *Lachnum* species. This reproducible difference suggests that caprylic acid (**3**) may serve as a useful chemotaxonomic marker under the examined culture conditions.

Overall, this study expands the chemical diversity known for *Erioscyphella* species and highlights the value of secondary metabolite profiling in both natural product discovery and fungal chemotaxonomy.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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