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Discovery of a new 1-monoglyceride from Streptomyces albogriseolus JM128 symbiotic with Lendenfeldia sponge

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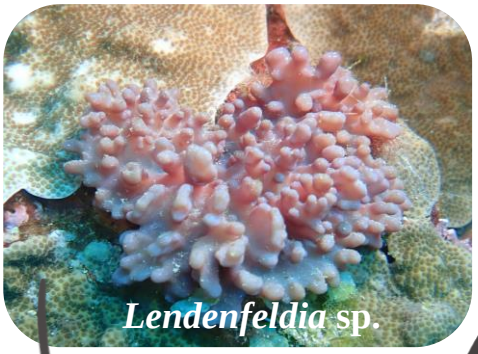
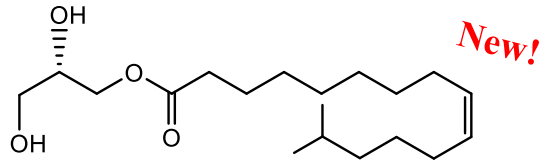
Abstract:	A new 1-monoglyceride derivative, (S)-2′,3′-dihydroxypropyl (Z)-14-methylpentadec-9-enoate (1), and a rare fatty acid derivative, 14-methylpentadecanoic acid (2), were isolated from the Gram-positive bacterium Streptomyces albogriseolus, which is symbiotic with the sponge identified as Lendenfeldia species. The structure of compound 2 was determined using single-crystal X-ray diffraction (SC-XRD) analysis. In contrast, the structure of compound 1 was confirmed through 2D NMR experiments combined with literature data. Both metabolites 1 and 2 exhibited inhibitory activity on nitric oxide (NO) release at a concentration of 10 μM.

Highlights

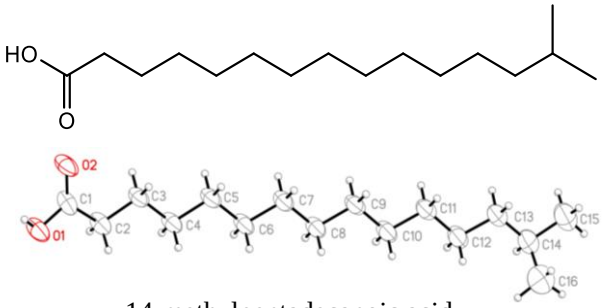
- A new monoglyceride **1** was isolated from *Streptomyces albogriseolus*.
- Structure of 14-methylpentadecanoic acid (**2**) confirmed by SC-XRD analysis.
- Metabolites **1** and **2** demonstrated inhibitory activity against NO release.

Graphical Abstract





(*S*)-2′,3′-dihydroxypropyl (*Z*)-14-methylpentadec-9-enoate



14-methylpentadecanoic acid

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Discovery of a new 1-monoglyceride from *Streptomyces albogriseolus* JM128 symbiotic with *Lendenfeldia* sponge

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ABSTRACT

A new 1-monoglyceride derivative, (*S*)-2′,3′-dihydroxypropyl (*Z*)-14-methylpentadec-9-enoate (**1**), and a rare fatty acid derivative, 14-methylpentadecanoic acid (**2**), were isolated from the Gram-positive bacterium *Streptomyces albogriseolus*, which is symbiotic with the sponge identified as *Lendenfeldia* species. The structure of compound **2** was determined using single-crystal X-ray diffraction (SC-XRD) analysis. In contrast, the structure of compound **1** was confirmed through 2D NMR experiments combined with literature data. Both metabolites **1** and **2** exhibited inhibitory activity on nitric oxide (NO) release at a concentration of 10 μM.

1. Introduction

Streptomyces are Gram-positive filamentous bacteria renowned for their prolific production of bioactive natural products (Sweeney et al., 2024). As part of our ongoing efforts to screen fermentation broths for novel marine-derived compounds (Chen et al., 2016, 2017), we identified an organic extract from strain JM128, which was taxonomically classified as *Streptomyces albogriseolus* (family *Streptomycetaceae*). This strain was originally isolated from bacterial communities associated with the marine sponge *Lendenfeldia* species (family *Thorectidae*).

Previous research has shown that metabolites from *S. albogriseolus* exhibit a broad spectrum of pharmacological activities, including antibacterial (Akshatha et al., 2022; Thirumurugan et al., 2018), anti-proliferative (Cui et al., 2007), cytotoxic (Ishida et al., 2022; Ma et al., 2020), and nematicidal effects (Zeng et al., 2013), as well as inhibitory activity against collagen proline hydroxylase (Ohta and Kamiya, 1981) and indoleamine 2,3-dioxygenase 1 (Gao et al., 2019).

In this study, we report the isolation of two metabolites from *S. albogriseolus*: a new 1-monoglyceride derivative, (*S*)-2′,3′-dihydroxypropyl (*Z*)-14-methylpentadec-9-enoate (**1**), and a rare fatty acid derivative, 14-methylpentadecanoic acid (also known as isopalmitic acid, **2**) (Ding et al., 2023; Richardson and Williams, 2013) (Fig. 1). The structure of **1** was elucidated through detailed spectroscopic analysis, while the structure of **2** was confirmed for the first time by SC-XRD analysis. Both compounds **1** and **2** exhibited inhibitory activity against NO production.

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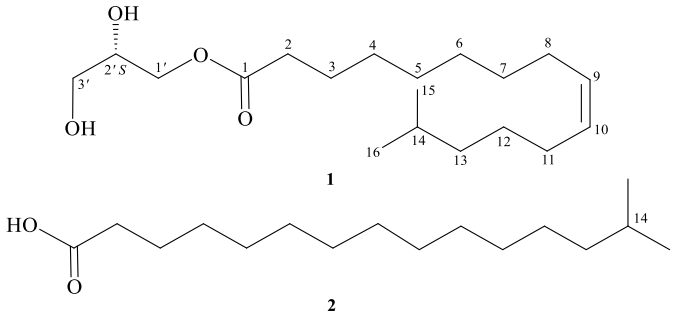

Fig. 1. Structures of (*S*)-2′,3′-dihydroxypropyl (*Z*)-14-methylpentadec-9-enoate (**1**) and 14-methylpentadecanoic acid (**2**).

2. Results and discussion

Monoglyceride **1** was isolated as an oil that gave an [M + Na]$^+$ ion peak at *m/z* 351.25046 in the HRESIMS, appropriate for a molecular formula of $C_{19}H_{36}O_4$ (calcd for $C_{19}H_{36}O_4$ + Na, 351.25058) (**Ω** =2). The IR spectrum of **1** showed characteristic absorption bands at 3375 and 1738 cm^{-1}, corresponding to hydroxy and ester groups, respectively. The ^{1}H and ^{13}C NMR data of **1** (Table 1) showed the presence of 19 carbon signals, which were identified by the assistance of a DEPT spectrum as two methyls, 12 sp^3 methylenes (including two oxymethylenes), two sp^3 methines (including one oxymethine), two sp^2 methines, and an ester carbonyl. DEPT and ^{13}C NMR spectra indicated a disubstituted olefin, evidenced by carbon signals at δ_C 130.1 (CH-9) and 129.7 (CH-10). These features were further supported by olefinic proton signals at δ_H 5.34 (2H, m, H-9 and H-10) in the ^{1}H NMR spectrum of **1** (Table 1). Additionally, a carbonyl peak at δ_C 174.3 (C-1) in the ^{13}C NMR spectrum indicated an ester group.

Table 1
^{1}H and ^{13}C NMR data for **1**.

C/H	δ_H[a] (*J* in Hz)	δ_C,[b] Mult.[c]
1		174.3, C
2	2.35 t (7.6)	34.1, CH_2
3	1.63 quint (7.6)	24.9, CH_2
4–6	1.26–1.34 m[d]	29.1, 3 × CH_2[g]
7	1.26–1.34 m[d]	29.7, CH_2
8	1.26–1.34 m[d]	27.1, CH_2
9	5.34 m[e]	130.1, CH
10	5.34 m[e]	129.7, CH
11	1.26–1.34 m[d]	27.5, CH_2
12	1.35 m[d]	27.4, CH_2
13	1.18 m	38.6, CH_2
14	1.53 nonet (6.8)	27.9, CH
15	0.87 d (6.8)[f]	22.6, CH_3
16	0.87 d (6.8)[f]	22.6, CH_3
1′	4.21 dd (11.6, 4.8); 4.15 dd (11.6, 6.0)	65.2, CH_2
2′	3.93 m	70.3, CH
3′	3.79 dd (11.6, 4.0); 3.60 dd (11.6, 6.0)	63.3, CH_2

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. [c] Multiplicity deduce by DEPT and HSQC spectra. [d,e,f,g] Signals overlapped.

The ^{1}H–^{1}H COSY spectrum of compound **1** revealed proton-proton couplings among H_2-2/H_2-3/H_2-4/H_2-5/H_2-6/H_2-7/H_2-8/H-9/H-10/H_2-11/H_2-12/H_2-13/H-14/H_3-15, as well as between H-14 and H_3-16, and among H_2-1′/H-2′/H_2-3′ (Fig. 2). These correlations were further confirmed through HMBC experiments. Notably, HMBC correlations between the oxymethylene protons H_2-1′ (δ_H 4.21 and 4.15) and the ester carbonyl carbon C-1 (δ_C 174.3) established the presence of an ester linkage in **1**. Comparison of the NMR data of **1** with those of known monoglycerides, (2*S*)-*α*-(7′*Z*,10′*Z*,13′*Z*)-hexadecatrienoic acid monoglyceride (**3**) and (2*S*)-*α*-(9′*Z*,12′*Z*,15′*Z*)-octadecatrienoic acid monoglyceride (**4**) (Ogihara et al., 2017), as well as the known fatty acid derivatives methyl oleate (Ruksilp, 2020; Wineburg and Swern, 1972) and (*Z*)-14-methyl-9-pentadecenoic acid (Carballeira et al., 2004) indicated that **1** is an esterification product of glycerol and (*Z*)-14-methyl-9-pentadecenoic acid.

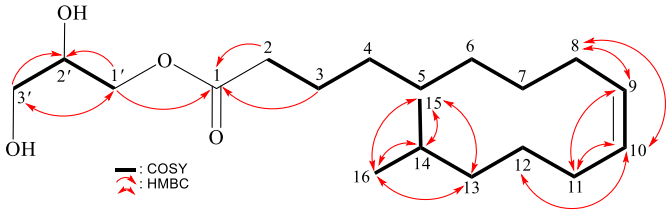

Fig. 2. Key COSY and HMBC correlations of **1**.

The absolute configuration of **1** was determined to be 2′*S*, based on the comparison of its positive optical rotation value with those of known compounds **3** and **4**, both of which possess a single stereogenic center. These results allowed for the unambiguous elucidation of the structure of **1**. Interestingly, the specific rotation values of 1-monoglyceride derivatives were found to be concentration-dependent, although they consistently remained within the same sign range (Chen et al., 2013; Nakao et al., 2024).

Based on spectroscopic analysis, a rarely reported fatty acid, 14-methylpentadecanoic acid (**2**), was identified (Ding et al., 2023; Richardson and Williams, 2013). To determine its structure for the first time, we conducted SC-XRD analysis, as illustrated in Fig. 3. Detailed crystal data and structure refinement parameters for **2** are presented in Table 2.

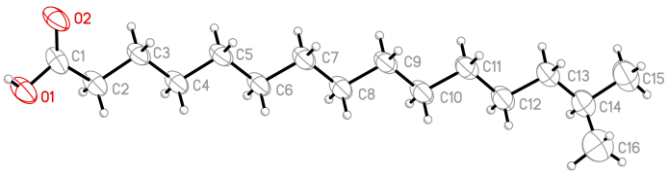

Fig. 3. The computer-generated ORTEP diagram of **2**.

Table 2
Crystal data and structure refinement details for **2**

Empirical formula	$C_{16}H_{32}O_2$
Formula weight	256.41
Temperature	200 (2) K
Wavelength	1.54178 Å
Crystal system	Triclinic
Space group	*P*1 (# 1)
Unit cell dimensions	*a* = 4.9022 (6) Å *α* = 87.384 (12)°
	b = 5.5746 (7) Å *β* = 85.549 (11)°
	c = 30.537 (5) Å *γ* = 76.526 (9)°
Volume	808.76 (19) Å^3
Z	2
Density (calculated)	1.053 Mg/m^3
Absorption coefficient	0.511 mm^{-1}
F (000)	288
Crystal size	0.413 × 0.255 × 0.022 mm^3
θ range for data collection	1.452 to 67.496°
Index ranges	$-5 \le h \le 5$, $-6 \le k \le 6$, $-36 \le l \le 36$
Reflection collected	14757
Independent reflections	2904 [*R* (int) = 0.2120]
Completeness to *θ* = 67.679°	99.8%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9798 and 0.6659
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2904/0/165
Goodness-of-fit on F^2	1.282
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.1407, wR_2 = 0.4038
R indices (all data)	R_1 = 0.2245, wR_2 = 0.4486
Extinction coefficient	n/a
Large diff. peak and hole	0.466 and −0.371 e.Å^{-3}

In this study, we evaluated the inhibitory effects of **1** and **2** on NO production in lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophages, and the results showed that **1** and **2** significantly inhibited NO production. Therefore, we propose that **1** and **2** possesses potential anti-inflammatory activity, possibly through the suppression of excessive NO production.

Table 3
Comparative NO inhibitory effects of test compounds in LPS-stimulated macrophages.

Compound	Concentration (μM)	Inhibition rate (%)
LPS	1 μg/mL	0
1	10	83.11 ± 4.37*
2	10	89.39 ± 3.93**
BHT	10	78.74 ± 5.71

RAW 264.7 macrophages were pretreated with each test compound for 1 hour, followed by stimulation with LPS (1 μg/mL) for 24 hours. NO levels in the culture supernatant were quantified using the Griess assay. The inhibition rate was calculated relative to the LPS-treated group, with the untreated control group serving as the baseline. Data are expressed as the mean ± SEM (n = 3). BHT (butylated hydroxytoluene, 10 μM) was used as a positive control. *p < 0.01, **p < 0.001 vs. BHT group.

3. Experimental

3.1. General

Optical rotation values were performed on a Jasco P-2000 digital polarimeter. IR spectra were obtained with a Thermo Scientific Nicolet iS5 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol ECZ NMR spectrometer at 400 MHz for ^{1}H NMR, and 100 MHz for ^{13}C NMR, respectively. Chemical shifts were reported in parts per million (δ), and the coupling constants (J) were expressed in Hertz (Hz). The residual peaks of the deuterated solvent, $CDCl_3$, were taken as reference points at δ_H 7.26 and δ_C 77.0 ppm, respectively. The ESIMS and HRESIMS spectra were acquired on a Thermo Fisher orbitrap Exploris 120 mass spectrometer equipped with an ESI ion source in positive ionization mode. Column chromatography (CC) was performed on C_{18} (17%) reverse phase silica gel (230–400 mesh, SiliCycle). TLC was performed on plates precoated with silica gel 60 F_{254} (Merck) and RP-18 W/UV_{254} (0.15-mm-thick, Macherey-Nagel), then sprayed with 10% H_2SO_4 solution followed by heating to visualize the spots. Normal-phase high performance liquid chromatography (NP-HPLC) was performed using a system comprised of a Hitachi L-7100 pump and a Rheodyne 7725i injection port with a normal-phase column (EF-SiO2, Catalog No.#: S/N E06210406, 5 μm, 120 Å, 250 × 20 mm, Galak).

3.2. Marine bacteria isolation, culture conditions, and extract preparation

The marine bacterium strain JM128 was isolated from the sponge *Lendenfeldia* sp., collected off the coast of Kenting, southern Taiwan, in April 2019 at a depth of 15 meters. Based on 16S rDNA gene sequence analysis, strain JM128 showed 100% identity to *Streptomyces albogriseolus* LBX2 (GenBank accession no. MN338058). The bacterial strain JM128 was cultivated on M1 agar medium composed of 8.0 g/L starch, 3.2 g/L yeast extract, 1.6 g/L peptone, and 14.4 g/L agar powder. The medium was prepared using a mixture of 640 mL seawater and 160 mL double-distilled water per liter and sterilized by autoclaving at 121 °C for 20 minutes. Following sterilization, JM128 was inoculated onto M1 agar plates by streaking and incubated statically at 25 °C for 5 days. Upon completion of incubation, agar sections containing visible bacterial colonies were aseptically excised using sterile instruments for extraction. The excised agar sections were transferred into separate extraction bottles containing ethyl acetate (EA) and subjected to ultrasonication for 30 minutes. After extraction, the organic solvent was removed using a rotary evaporator to yield crude extracts, which were subsequently stored for further analysis.

3.3. Separation

The crude extract (4.93 g) was subjected to separation by C_{18} reverse-phase silica gel column chromatography (RP-Si CC), eluted with a dichloromethane/water mixture (DCM/H_2O, 1:6), resulting in 10 fractions A~J. Fraction C was further purified using C_{18} RP-Si CC with methanol (MeOH) as the mobile phase, yielding nine subfractions C1~C9. Fraction C6 was then purified again using C_{18} RP-Si CC, employing an acetonitrile/water mixture (9:1) as the mobile phase to produce eight subfractions C6A~C6H. From fraction C6F, compound **1** (8.18 mg) was isolated *via* NP-HPLC using an *n*-hexane/ acetone mixture (3:1) as the mobile phase.

Similarly, fraction D was further purified by C_{18} RP-Si CC using an acetonitrile/water mixture (9:1), resulting in subfractions D1–D10. Fraction D10 was then subjected to NP-HPLC using an *n*-hexane/acetone mixture (3:1), affording compound **2** (2.0 mg).

3.3.1. (S)-2′,3′-Dihydroxypropyl (Z)-14-methylpentadec-9-enoate (1)

Colorless oil; $[\alpha]_D$ +101 (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3375, 1738 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data, see Table 1. ESIMS: m/z 351 $[M + Na]^+$, HRESIMS: m/z 351.25046 (calcd. for $C_{19}H_{36}O_4$ + Na, 351.25058).

3.4. SC-XRD of 14-methylpentadecanoic acid (2)

Prismatic crystals of compound **2** were obtained by slow evaporation from an acetone solution at 4 °C. A crystal specimen measuring 0.413 × 0.255 × 0.022 mm³ was determined to crystallize in the triclinic system with space group $P1$ (# 1) (Hestenes and Holt, 2007). X-ray diffraction data were collected on a Bruker D8 Venture diffractometer using Cu Kα radiation. The structure was solved by direct methods and refined using a full-matrix least-squares approach (Sheldrick, 2015a, 2015b). Crystallographic data for **2** have been deposited with the Cambridge Crystallographic Data Centre (CCDC) under accession number CCDC 2412836. These data are available from the CCDC via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or by contacting the CCDC at 12 Union Road, Cambridge CB2 1EZ, UK, or by email at deposit@ccdc.cam.ac.uk.

3.5. Cell culture and reagents

The murine macrophage cell line RAW264.7 was obtained from the American Type Culture Collection (ATCC, TIB-71; Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, USA) and 1% penicillin-streptomycin. Cells were maintained at 37 °C in a humidified incubator containing 5% CO_2.

3.6. NO Inhibition assay

RAW264.7 cells were seeded in 96-well plates at a density of 1 × 10^5 cells/well and allowed to adhere overnight. The cells were then stimulated with lipopolysaccharide (LPS; *Escherichia coli* O111:B4, Sigma-Aldrich) at a final

concentration of 1 μg/mL for 1 hour. Following stimulation, cells were treated with the test sample at a concentration of 10 μM for 24 hours. A group treated with butylated hydroxytoluene (BHT; 10 μM) served as the positive control. After the incubation period, culture supernatants were collected, and NO levels were quantified using the Griess reagent (Promega, WI, USA) according to the manufacturer's protocol. Absorbance was measured at 540 nm using a microplate reader. The percentage inhibition of NO production was calculated using the formula: NO inhibition rate (%) = [(LPS − sample) /(LPS − control)] × 100. All experiments were performed in triplicate, and data are presented as the mean ± standard deviation (SD).

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2025.xx.xxxx

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