Neomastoidin A, a Novel Monoacylglycerol with an Amino Acid Moiety from *Macrolepiota neomastoidea*

Ki Hyun Kim,[1] Sang Un Choi,[2] and Kang Ro Lee*[1]
[1]*Natural Products Laboratory, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea*
[2]*Korea Research Institute of Chemical Technology, Teajeon 305-600, Korea*

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A novel monoacylglycerol with an amino acid moiety, neomastoidin A (**1**), was isolated from the fruiting bodies of the poisonous mushroom *Macrolepiota neomastoidea*. The structure of **1** was established by extensive spectroscopic analysis and further confirmed by application of a dibenzoate chirality method. Neomastoidin A (**1**) exhibited cytotoxicity against SK-OV-3 and SK-MEL-2 cell lines.

In our continuing study of Korean poisonous mushroom *Macrolepiota neomastoidea* (Agaricaceae),[1] we isolated further an unusual monoacylglycerol from a MeOH extract of the fruiting bodies of *M. neomastoidea*, which causes severe gastrointestinal symptoms.[2] Previous chemical investigation of this mushroom reported the isolation of several alkaloids.[2]

We describe herein the isolation, structure determination, and bioactivity of an unprecedented monoacylglycerol with an *O*-methyl-L-serine moiety, neomastoidin A (**1**) (Chart 1), from the MeOH extract of *M. neomastoidea*. The structure, including the absolute stereochemistry of **1**, was elucidated by spectroscopic methods and CD data analysis.

The fresh fruiting bodies of *M. neomastoidea* (air-dried weight 132 g), collected at Mt. Jiri, Namwon of Jeonbuk province, Korea, were powdered and extracted with 80% MeOH at room temperature. The resulting residue (21.4 g) was partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, successively. The $CHCl_3$ soluble fraction (283 mg) was separated on a silica gel Lobar A®-column eluting with $CHCl_3$–MeOH (10:1) to yield fractions 1–7. Fraction C7 was purified by normal phase HPLC to afford **1** (8 mg, $CHCl_3$–MeOH, 20:1).

Neomastoidin A (**1**) was obtained as a colorless gum, $[\alpha]_D^{25}$ +24.5 (*c* 0.35, MeOH). The ion at *m/z* 456 ($[M + H]^+$) in the FABMS was in agreement with the molecular formula $C_{25}H_{45}NO_6$ (4 degrees of unsaturation), which was confirmed by the positive HRFABMS ($[M + Na]^+$ ion at *m/z* 478.3151, calcd for $C_{25}H_{45}NO_6$ + Na, 478.3145). Its IR spectrum exhibited the presence of hydroxy (3396 cm^{-1}), ester carbonyl, (1735 cm^{-1}), and double bond units (1657 cm^{-1}). The ^1H NMR spectrum of **1** displayed signals for the presence of a terminal methyl proton at δ 0.90 (3H, t, $J = 6.5$ Hz), methylene protons at δ 1.37–1.25 (br s), and signals of two olefinic bonds at δ 5.34 (4H, m). Four resonances observed at δ 5.22 (1H, m), 4.43 (1H, dd, $J = 4.0$, 12.0 Hz), 4.20 (1H, dd, $J = 7.0$, 12.0 Hz), and 4.00 (2H, m) confirmed the presence of a glycerol moiety. These data indicated that **1** possessed an aliphatic long chain containing two double bonds and a glycerol moiety. The ^{13}C NMR data of **1** showed one quaternary carbon at δ 173.4 (C=O), four olefinic methine carbons at δ 129.6, 129.5, 127.9, and 127.8 (C=C), resonances for aliphatic chains at δ 29.6–29.0 (CH_2), and one methyl carbon at δ 13.2 (CH_3), as well as signals of a glycerol moiety at δ 70.6 (CHOH), 63.7 (CH_2OH), and 62.5 (CH_2OH). The stereochemistries at C-9′/C-10′ and C-12′/C-13′ were assigned as 9′(Z), and 12′(Z), respectively, since the geometry (Z) of the double bond in the unsaturated long chain was determined on the basis of the ^{13}C NMR chemical shifts of C-8′ and C-14′ (δ 27.1 and 27.4) of the methylene carbon next to the olefinic carbon, which usually appears at δ ca. 27 in Z isomers and at δ ca. 30 in E isomers.[3] The above spectral data and literature enabled the structure of **1** to be established as 1-*O*-linoleoylglycerol.[4] In addition, the ^{13}C and ^1H NMR data showed resonances for one quaternary carbon at δ 173.7, a methine bonded to nitrogen at δ 59.3 (δ 4.27), a hydroxymethylene at δ 66.3 (δ 3.64 and 3.18), and one methoxy group at δ 53.5 (δ 3.22), suggesting that **1** also contained an *O*-methylserine.[5] All these spectral data revealed that **1** was 1-*O*-linoleoylglycerol with *O*-methylserine.

The gross structure of **1** was further confirmed by 2D NMR studies. In the ^1H–^1H COSY spectrum (Figure 1), the hydroxymethylene at δ 4.43 and 4.20 (H-1) exhibited correlation with the oxygenated methine proton at δ 5.22 (H-2), which coupled further to the signal at δ 4.00 (H-3). Olefinic protons at δ 5.34 (H-9′, -10′, -12′, and -13′) showed coupling with methylene protons resonating at δ 2.02 (H-8′ and -14′) and 2.77 (H-11′). Additionally, the methine proton at δ 4.27 (H-2″) showed coupling with the oxygenated methylene at δ 3.64 (H-3″). In the HMBC spectrum (Figure 1), the proton at δ 4.43 (H-1a) exhibited correlations with the carbonyl at δ 173.4 (C-1′), the oxygenated methine at δ 70.6 (C-2), and the hydroxymethylene at δ 63.7 (C-3). In the HMBC spectrum the methine proton adjacent to NH_2 at δ 4.27 (H-2″) correlated with the oxygenated methine at δ 70.6 (C-2). Furthermore, a methoxy group at δ 3.22 correlat-

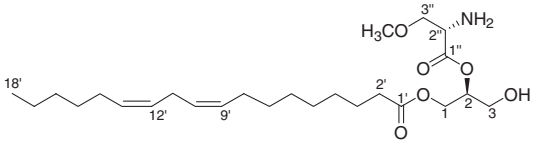

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Chart 1.

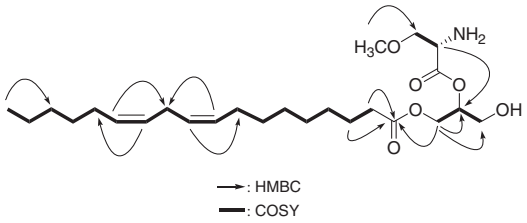
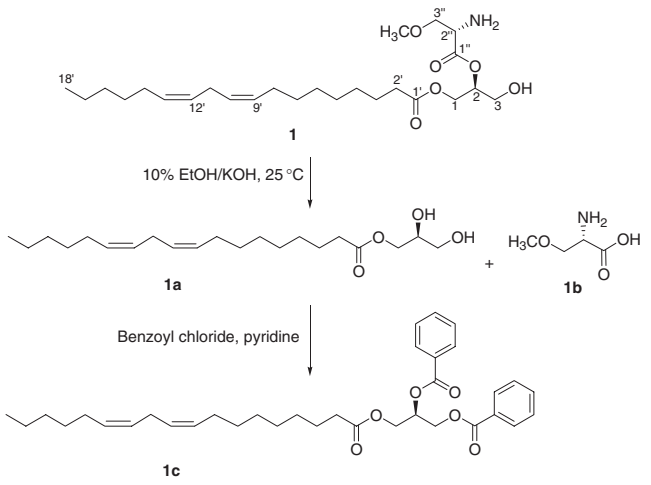


Figure 1. Key HMBC and ^1H–^1H COSY correlations of **1**.



Scheme 1. Hydrolysis of **1** and synthesis of **1c**.

ed with the hydroxymethylene at δ 66.3 (C-3″). The above COSY and HMBC correlations confirmed the structure of **1** to be 1-*O*-linoleoyl-2-*O*-(*O*-methyl-L-seryl)glycerol.

Alkaline hydrolysis of **1** (Scheme 1) produced a long chain fatty acid **1a**, which was identified as 1-*O*-linoleoylglycerol by comparison of ^{1}H NMR data.[4] The remaining amino acid moiety **1b** was suggested to be *O*-methylserine based on the results of the ^{1}H NMR analysis.[5,6] Both these segments were also confirmed by the ion peaks at m/z 294 ($C_{19}H_{34}O_2$) and 119 ($C_4H_9NO_3$) in the FABMS spectrum of **1a** and **1b**, respectively. Besides, hydrolysis of **1** afforded free fatty acid residue as a by-product. The absolute stereochemistry of C-2 was determined using a dibenzoate chirality method.[7] The hydroxy groups at C-2 and C-3 of **1a** were esterified with benzoyl chloride in dry pyridine to obtain **1c** (Scheme 1).[8] The CD spectrum of **1c** exhibited a positive exciton couplet CD peak at 235 nm to reflect its 2S configuration.[7] The optical rotation of **1a**; $[\alpha]_D^{25}$ +4.7 (*c* 0.07, MeOH) was also in agreement with that of a synthetic monoglyceride in the S form.[4] The absolute configuration of *O*-methylserine was determined to be in the L-form by the measurement of optical rotation of **1b**; $[\alpha]_D^{25}$ +9.8 (*c* 0.10, MeOH).[5] Thus, the structure of **1** was established as 1-*O*-linoleoyl-(2*S*)-*O*-(*O*-methyl-L-seryl)glycerol, and designated to be neomastoidin A.[9]

A literature survey revealed that compounds of this type are seldom reported from both natural sources and synthesized compounds.[10] The cytotoxicity of **1** was evaluated against the A549 (non small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT (colon adenocarcinoma) human tumor cell lines in vitro using the SRB assay.[11] Compound **1** exhibited cytotoxicity against SK-OV-3 and SK-MEL-2 cells (IC_{50} 41.6 and 26.5 μM), while compound **1** was inactive ($IC_{50} > 50$ μM) toward other two tumor cell lines.

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References and Notes

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6 **1b** white solid; $[\alpha]_D^{25}$ +9.8 (*c* 0.10, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 4.31 (1H, m, H-2), 3.95 (1H, m, H-3a), 3.82 (1H, m, H-3b), 3.40 (3H, s, CH_3-O); FABMS m/z 119 $[M]^+$.
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8 **1c** colorless oil; CD (MeOH) $[\theta]_{219}$ −2300, $[\theta]_{235}$ +8200; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.95 (3H, t, $J = 7.0$ Hz, H-18′), 1.30–1.37 (14H, br s, H-4′, -5′, -6′, -7′, -15′, -16′, and -17′), 1.63 (2H, m, H-3′), 2.07 (4H, m, H-8′ and -14′), 2.38 (2H, t, $J = 7.5$ Hz, H-2′), 2.82 (2H, t, $J = 6.5$ Hz, H-11′), 4.25 (1H, dd, $J = 7.0$, 12.0 Hz, H-1a), 4.47 (1H, dd, $J = 4.0$, 12.0 Hz, H-1b), 4.54–4.60 (2H, m, H-3), 5.07 (1H, m, H-2), 5.32–5.36 (4H, m, H-9′, -10′, -12′, and -13′), 7.49–7.52 (4H, m, Ar–H), 7.60–7.63 (2H, m, Ar–H), 8.01–8.02 (4H, m, Ar–H); FABMS m/z: 562 $[M]^+$.
9 Compound **1** colorless gum; $[\alpha]_D^{25}$ +24.5 (*c* 0.35, MeOH); IR (KBr) ν_{max} 3396, 1735, 1657, 1520, 1018, 777 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ 0.90 (3H, t, $J = 6.5$ Hz, H-18′), 1.25–1.37 (14H, br s, H-4′, -5′, -6′, -7′, -15′, -16′, and -17′), 1.60 (2H, m, H-3′), 2.02 (4H, m, H-8′ and 14′), 2.30 (2H, t, $J = 7.5$ Hz, H-2′), 2.77 (2H, t, $J = 6.5$ Hz, H-11′), 3.18 (1H, m, H-3″a), 3.22 (3H, s, 3″-OCH_3), 3.64 (1H, m, H-3″b), 4.00 (2H, m, H-3), 4.20 (1H, dd, $J = 7.0$, 12.0 Hz, H-1a), 4.27 (1H, m, H-2″), 4.43 (1H, dd, $J = 4.0$, 12.0 Hz, H-1b), 5.22 (1H, m, H-2), 5.32–5.36 (4H, m, H-9′, -10′, -12′, and -13′); ^{13}C NMR (125 MHz, CD_3OD): δ 13.2 (C-18′), 22.4 (C-17′), 24.8 (C-3′), 25.3 (C-11′), 27.1 (C-8′), 27.4 (C-14′), 29.0–29.6 (C-4′, -5′, -6′, -7′, and -15′), 31.9 (C-16′), 33.7 (C-2′), 53.5 (OCH_3), 59.3 (C-2″), 62.5 (C-1), 63.7 (C-3), 66.3 (C-3″), 70.6 (C-2), 127.8 (C-10′), 127.9 (C-12′), 129.5 (C-9′), 129.6 (C-13′), 173.4 (C-1′), 173.7 (C-1″); FABMS m/z: 456 $[M + H]^+$; HRFABMS (positive-ion mode) m/z: 478.3151 $[M + Na]^+$ (calcd for $C_{25}H_{45}NO_6$ + Na, 478.3145).
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