

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

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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),<sup>1</sup> we isolated further an unusual monoacylglycerol from a MeOH extract of the fruiting bodies of *M. neomastoidea*, which causes severe gastrointestinal symptoms.<sup>2</sup> Previous chemical investigation of this mushroom reported the isolation of several alkaloids.<sup>2</sup>

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<sub>3</sub>, and *n*-BuOH, successively. The CHCl<sub>3</sub> soluble fraction (283 mg) was separated on a silica gel Lobar A<sup>®</sup>-column eluting with CHCl<sub>3</sub>-MeOH (10:1) to yield fractions 1–7. Fraction C7 was purified by normal phase HPLC to afford **1** (8 mg, CHCl<sub>3</sub>-MeOH, 20:1).

Neomastoidin A (**1**) was obtained as a colorless gum, [ $\alpha$ ]<sub>D</sub><sup>25</sup> +24.5 (*c* 0.35, MeOH). The ion at *m/z* 456 ([M + H]<sup>+</sup>) in the FABMS was in agreement with the molecular formula C<sub>25</sub>H<sub>45</sub>NO<sub>6</sub> (4 degrees of unsaturation), which was confirmed by the positive HRFABMS ([M + Na]<sup>+</sup> ion at *m/z* 478.3151, calcd for C<sub>25</sub>H<sub>45</sub>NO<sub>6</sub> + Na, 478.3145). Its IR spectrum exhibited the presence of hydroxy (3396 cm<sup>-1</sup>), ester carbonyl, (1735 cm<sup>-1</sup>), and double bond units (1657 cm<sup>-1</sup>). The <sup>1</sup>H NMR spectrum of **1** displayed signals for the presence of a terminal methyl proton at  $\delta$  0.90 (3H, t, *J* = 6.5 Hz), methylene protons at  $\delta$  1.37–1.25 (br s), and signals of two olefinic bonds at  $\delta$

5.34 (4H, m). Four resonances observed at  $\delta$  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 <sup>13</sup>C NMR data of **1** showed one quaternary carbon at  $\delta$  173.4 (C=O), four olefinic methine carbons at  $\delta$  129.6, 129.5, 127.9, and 127.8 (C=C), resonances for aliphatic chains at  $\delta$  29.6–29.0 (CH<sub>2</sub>), and one methyl carbon at  $\delta$  13.2 (CH<sub>3</sub>), as well as signals of a glycerol moiety at  $\delta$  70.6 (CHOH), 63.7 (CH<sub>2</sub>OH), and 62.5 (CH<sub>2</sub>OH). 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 <sup>13</sup>C NMR chemical shifts of C-8' and C-14' ( $\delta$  27.1 and 27.4) of the methylene carbon next to the olefinic carbon, which usually appears at  $\delta$  ca. 27 in Z isomers and at  $\delta$  ca. 30 in E isomers.<sup>3</sup> The above spectral data and literature enabled the structure of **1** to be established as 1-*O*-linoleoylglycerol.<sup>4</sup> In addition, the <sup>13</sup>C and <sup>1</sup>H NMR data showed resonances for one quaternary carbon at  $\delta$  173.7, a methine bonded to nitrogen at  $\delta$  59.3 ( $\delta$  4.27), a hydroxymethylene at  $\delta$  66.3 ( $\delta$  3.64 and 3.18), and one methoxy group at  $\delta$  53.5 ( $\delta$  3.22), suggesting that **1** also contained an *O*-methylserine.<sup>5</sup> 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 <sup>1</sup>H-<sup>1</sup>H COSY spectrum (Figure 1), the hydroxymethylene at  $\delta$  4.43 and 4.20 (H-1) exhibited correlation with the oxygenated methine proton at  $\delta$  5.22 (H-2), which coupled further to the signal at  $\delta$  4.00 (H-3). Olefinic protons at  $\delta$  5.34 (H-9', -10', -12', and -13') showed coupling with methylene protons resonating at  $\delta$  2.02 (H-8' and -14') and 2.77 (H-11'). Additionally, the methine proton at  $\delta$  4.27 (H-2'') showed coupling with the oxygenated methylene at  $\delta$  3.64 (H-3''). In the HMBC spectrum (Figure 1), the proton at  $\delta$  4.43 (H-1a) exhibited correlations with the carbonyl at  $\delta$  173.4 (C-1'), the oxygenated methine at  $\delta$  70.6 (C-2), and the hydroxymethylene at  $\delta$  63.7 (C-3). In the HMBC spectrum the methine proton adjacent to NH<sub>2</sub> at  $\delta$  4.27 (H-2'') correlated with the oxygenated methine at  $\delta$  70.6 (C-2). Furthermore, a methoxy group at  $\delta$  3.22 correlated

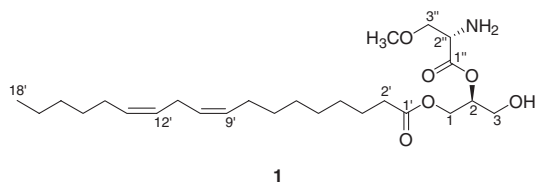


Chart 1.

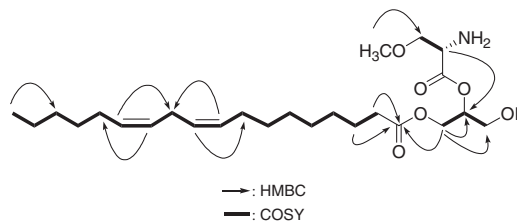
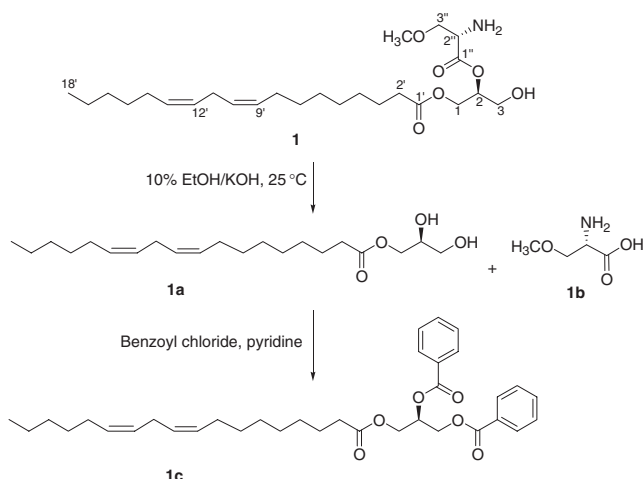


Figure 1. Key HMBC and <sup>1</sup>H-<sup>1</sup>H COSY correlations of **1**.



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

ed with the hydroxymethylene at  $\delta$  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  $^1\text{H NMR}$  data.<sup>4</sup> The remaining amino acid moiety **1b** was suggested to be *O*-methylserine based on the results of the  $^1\text{H NMR}$  analysis.<sup>5,6</sup> Both these segments were also confirmed by the ion peaks at  $m/z$  294 ( $\text{C}_{19}\text{H}_{34}\text{O}_2$ ) and 119 ( $\text{C}_4\text{H}_9\text{NO}_3$ ) 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.<sup>7</sup> The hydroxy groups at C-2 and C-3 of **1a** were esterified with benzoyl chloride in dry pyridine to obtain **1c** (Scheme 1).<sup>8</sup> The CD spectrum of **1c** exhibited a positive exciton couplet CD peak at 235 nm to reflect its 2S configuration.<sup>7</sup> The optical rotation of **1a**;  $[\alpha]_{\text{D}}^{25} +4.7$  ( $c$  0.07, MeOH) was also in agreement with that of a synthetic monoglyceride in the S form.<sup>4</sup> The absolute configuration of *O*-methylserine was determined to be in the L-form by the measurement of optical rotation of **1b**;  $[\alpha]_{\text{D}}^{25} +9.8$  ( $c$  0.10, MeOH).<sup>5</sup> 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.<sup>9</sup>

A literature survey revealed that compounds of this type are seldom reported from both natural sources and synthesized compounds.<sup>10</sup> 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.<sup>11</sup> Compound **1** exhibited cytotoxicity against SK-OV-3 and SK-MEL-2 cells ( $\text{IC}_{50}$  41.6 and 26.5  $\mu\text{M}$ ), while compound **1** was inactive ( $\text{IC}_{50} > 50 \mu\text{M}$ ) toward other two tumor cell lines.

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- 6 **1b** white solid;  $[\alpha]_{\text{D}}^{25} +9.8$  ( $c$  0.10, MeOH);  $^1\text{H NMR}$  (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  4.31 (1H, m, H-2), 3.95 (1H, m, H-3a), 3.82 (1H, m, H-3b), 3.40 (3H, s,  $\text{CH}_3\text{-O}$ ); FABMS  $m/z$  119  $[\text{M}]^+$ .
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- 8 **1c** colorless oil; CD (MeOH)  $[\theta]_{219} -2300$ ,  $[\theta]_{235} +8200$ ;  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  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  $[\text{M}]^+$ .
- 9 Compound **1** colorless gum;  $[\alpha]_{\text{D}}^{25} +24.5$  ( $c$  0.35, MeOH); IR (KBr)  $\nu_{\text{max}}$  3396, 1735, 1657, 1520, 1018, 777  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  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<sub>3</sub>), 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');  $^{13}\text{C NMR}$  (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  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<sub>3</sub>), 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  $[\text{M} + \text{H}]^+$ ; HRFABMS (positive-ion mode)  $m/z$ : 478.3151  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{25}\text{H}_{45}\text{NO}_6 + \text{Na}$ , 478.3145).
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