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(−)-Ternatin, a highly N-methylated cyclic heptapeptide that inhibits fat accumulation: structure and synthesis

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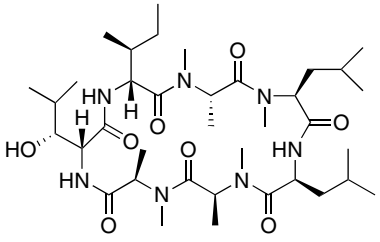
Abstract—A highly N-methylated cyclic heptapeptide, (−)-ternatin, was isolated from the mushroom *Coriolus versicolor*, which significantly suppressed fat accumulation against 3T3-L1 murine adipocytes ($EC_{50} = 0.14$ μg/mL). Although ternatin was previously reported to be antibacterial or antimicrobial compound, its inhibitory effect on fat accumulation has been first shown. The structure of (−)-ternatin was revised to be a cyclo [D-*allo*-Ile[1]-L-(NMe)Ala[2]-L-(NMe)Leu[3]-L-Leu[4]-L-(NMe)Ala[5]-D-(NMe)Ala[6]-(2*R*,3*R*)-3-hydroxy-Leu[7]] by spectroscopic analysis and chemical synthesis.

Obesity caused by a modern lifestyle, such as an excessive intake of food and a lack of sleep and exercise, is a serious problem worldwide.[1] WHO has reported that at least 300 million adults are clinically obese. Obesity can often trigger serious lifestyle-related diseases such as cancer, cardiovascular disease, hypertension, hyperlipemia, and diabetes. Recently, a variety of foods have been highlighted as being low-calorie and may confer some benefits to the body. From the point of view, mushrooms may be one of these perfect candidates. In our continuing search for bioactive metabolites from mushrooms,[2] we found that the highly N-methylated cyclic heptapeptide (−)-ternatin (**1**), which were isolated from the mushroom *Coriolus versicolor*, potently inhibited fat accumulation against 3T3-L1 murine adipocytes (Fig. 1). We report here the structure and synthesis of **1**.



Ternatin (**1**)

Figure 1. Structure of ternatin (**1**).

The mushroom *C. versicolor* (5 kg) was homogenized and extracted with 80% aqueous ethanol followed by partition with EtOAc and H_2O. The EtOAc layer potently inhibited fat accumulation against 3T3-L1 murine adipocytes, which was partitioned with aqueous methanol and hexane. The aqueous methanol layer was separated by silica-gel and Sephadex LH-20 gel permeation column chromatography followed by reversed-phase HPLC to afford a cyclic heptapeptide, (−)-ternatin (**1**) [11.5 mg; $[\alpha]_D^{20}$ − 23.5 (*c* 0.13, EtOH)]. Compound **1** inhibited fat accumulation against 3T3-L1 murine adipocytes that were differentiated from preadipocytes by treatment with insulin ($EC_{50} = 0.14$ μg/mL).[4–6] Meanwhile, cell viability was not greatly affected at the EC_{50} value.[3] Thus, ternatin (**1**) was considered to be a potential anti-obesity drug.

The molecular formula of **1** was found to be $C_{37}H_{67}N_7O_8$ [$(M+Na)^+$, *m/z* 760.4945, Δ −0.4 mmu] by HRFABMS. Detailed analysis of ^1H, ^{13}C NMR, HMBC, and COSY spectra in benzene-d_6 gave the

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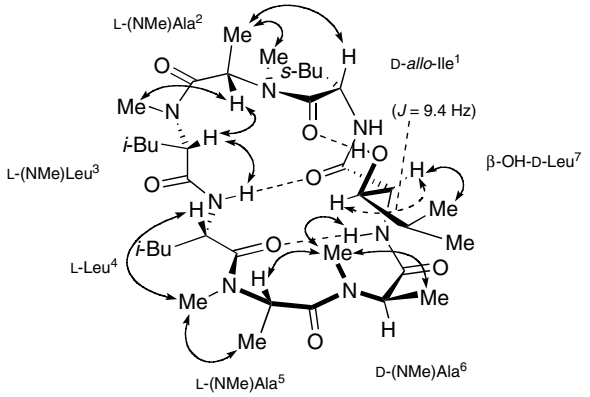
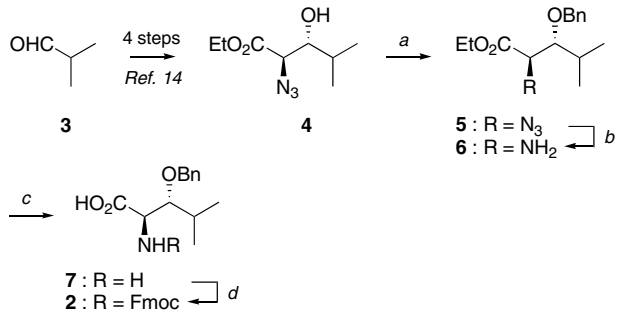


Figure 2. Plausible stereostructures of ternatin (**1**) based on 2D NMR spectra. Arrows indicate ROESY correlations. Possible intramolecular bonds between OH or NH protons and carbonyl oxygen atoms are shown by dotted lines.

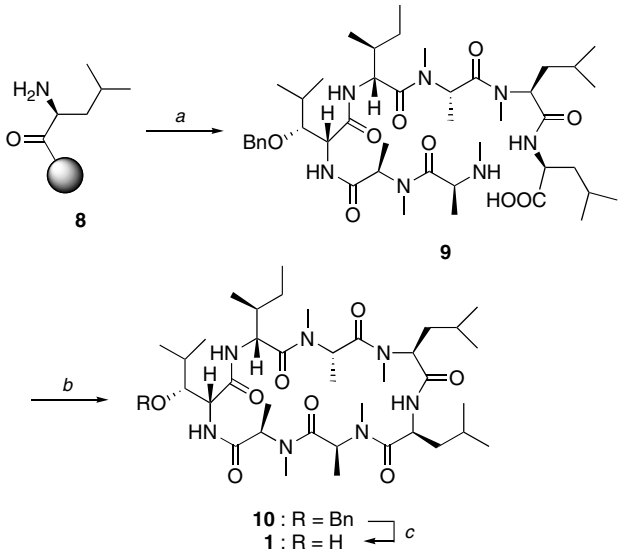
planar structure of **1**. Amino acid analysis using the Marfey method[7,8] revealed that **1** was composed of L-(NMe)Leu[3], L-Leu[4], and both L- and D-(NMe)Ala[2,5,6]. A hydroxyl proton and two amide NH protons in the L-Leu[4] and β-OH-Leu[7] moieties were observed in the ^{1}H NMR spectrum both in CD_3OD and in C_6D_6, which suggested that (−)-ternatin (**1**) had a single conformation that was fixed by three intramolecular hydrogen bonds in these solvents. Detailed analysis of ROESY correlations and coupling constants elucidated a plausible stereostructure shown in Figure 2. The absolute stereochemistry of (NMe)Ala[2] was confirmed to be L. The coupling constant of the two methine protons H-2 and H-3 in β-OH-Leu[7] was 9.4 Hz, which suggested an *anti* arrangement. Thus, the absolute stereochemistry of β-OH-Leu[7] was confirmed to be 2*R*,3*R*. As for the (NMe)Ala[5,6] moiety, the stereoisomer L-(NMe)Ala[5]-D-(NMe)Ala[6] was considered as a plausible conformer based on ROESY correlations. Likewise, the stereochemistry of Ile[1] was suggested to be D- or D-*allo*-form. As a result, the structure of **1** was found to be identical to that of ternatin, which was previously isolated as an antibacterial or antimicrobial compound from the fungus *Didymocladium ternatum*[9] and *Cladobotryum* sp.[10] The stereochemistry of Ile[1] moiety of ternatin was previously described as D-form,[9,11] but its X-ray crystal structure has suggested D-*allo*-form.[9,12] Furthermore, the sign of optical rotation of ternatin was not clearly mentioned.[13] Thus, it was still ambiguous whether (−)-**1** was identical to authentic compound. To confirm the stereochemistry and biological activities of **1**, synthetic study was carried out.

First, Fmoc-β-OBn-D-Leu-OH (**2**) was prepared (Scheme 1). Isobutyraldehyde (**3**) was converted to the chiral secondary alcohol **4** as described in the literature.[14] The hydroxyl group in **4** was protected with a Bn group followed by the Pd/C(en)-mediated selective hydrogenation[15] of azide to afford amine **6**. Hydrolysis of the ester and Fmoc-protection of the amino group gave **2**.

A cyclic peptide **1** was then synthesized in the solid phase (Scheme 2). Starting from Trt(2-Cl) resin pre-



Scheme 1. Reagents and Conditions: (a) benzyl-2,2,2-trichloroacetimidate, TfOH, CH_2Cl_2, rt, 57% (recovered 43% of **4**); (b) H_2, Pd/C(en), MeOH, rt, 70%; (c) 1 M NaOH aq, THF, rt, 89%; (d) Fmoc–ONSu, 9% Na_2CO_3 aq, DMF, 0 °C to rt, 89%.



Scheme 2. Reagents and Conditions: (a) solid-phase peptide synthesis (Fmoc deprotection, 20% piperidine in DMF; coupling reaction, HATU, DMF; cleavage from the resin, 0.5% TFA/CH_2Cl_2, rt), 70%; (b) HATU, HOAt, *i*-Pr_2NEt, CH_2Cl_2, 0 °C to rt, 73%; (c) H_2, 10% Pd/C, MeOH, rt, 81%.

loaded with L-Leu[4] [H-L-Leu-Trt(2-Cl) resin (**8**)], incorporation was elaborated in the [C] to [N] direction by sequential deprotection with 20% piperidine followed by HATU-mediated coupling with synthesized leucine derivative **2** and the corresponding Fmoc amino acids. Deprotection of the final Fmoc group and cleavage from the resin under a mild acidic condition gave linear heptapeptide **9** in 70% yield. Macrolactamization of **9** was performed using HATU, HOAt and *i*-Pr_2NEt in dilute solution (0.77 mM).[16] Finally, removal of the Bn group in lactam **10** afforded cyclic heptapeptide **1**.[17]

The ^{1}H NMR data of synthetic ternatin was identical to that of natural (−)-**1**. The optical rotation of **1** was −35.5° (*c* 0.40, EtOH), which agreed with that of natural compound. Synthetic ternatin (**1**) potently inhibited the adipogenesis in 3T3-L1 cells (Fig. 3). Two stereoisomers cyclo [D-Ile[1]-L-(NMe)Ala[2]-L-(NMe)Leu[3]-L-Leu[4]-L-(NMe)Ala[5]-D-(NMe)Ala[6]-(2*R*,3*R*)-3-hydroxy-Leu[7]]- and cyclo [D-Ile[1]-L-(NMe)Ala[2]-L-(NMe)Leu[3]-L-Leu[4]-D-

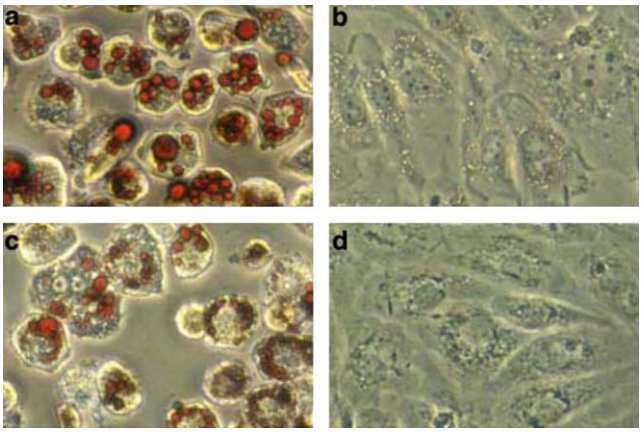


Figure 3. Effects of synthetic ternatin (**1**) on the fat accumulation of 3T3-L1 murine adipocytes. **a** and **c**, Differentiated 3T3-L1 murine adipocytes induced by insulin or rosiglitazone (incubated for 11 days). The accumulated fat was stained with oil red O. **b** and **d**, incubated 3T3-L1 adipocytes as with **a** and **c** in the presence of **1** (1 μg/ml).

(NMe)Ala^5-L-(NMe)Ala^6-(2*R*,3*R*)-3-hydroxy-Leu^7] were also synthesized. However, these spectroscopic data were not identical to that of natural **1**. Thus, the structure of **1** was confirmed to be a cyclo [D-*allo*-Ile^1-L-(NMe)Ala^2-L-(NMe)Leu^3-L-Leu^4-L-(NMe)Ala^5-D-(NMe)Ala^6-(2*R*,3*R*)-3-hydroxy-Leu^7] as shown in Figure 1., being identical to the reported X-ray crystal structure. It is reasonable to suppose that the previous assignment of D-Ile^1 moiety in ternatin was incorrect.

In summary, (−)-ternatin (**1**) was isolated from the mushroom *C. versicolor*, which significantly inhibited fat accumulation against 3T3-L1 murine adipocytes. The structure of **1** was determined by spectroscopic analysis and chemical synthesis. The fat accumulation-inhibitory effect of **1** may be caused by the prevention of adipogenesis and/or the activation of lipogenesis in adipocytes. Further studies on the biological activities of ternatin and its derivatives are in progress.

Acknowledgements

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References and notes

1. Recent selected reviews on obesity: (a) Campfield, L. A.; Smith, F. J.; Burn, P. *Science* **1998**, *280*, 1383; (b) Kolonin, M. G.; Saha, P. K.; Chan, L.; Pasqualini, R.; Arap, R. *Nat. Med.* **2004**, *10*, 625; (c) Calle, E. E.; Kaaks, R. *Nat. Rev. Cancer* **2004**, *4*, 579; (d) Evans, R. M.; Barish, G. D.; Wang, Y. X. *Nat. Med.* **2004**, *10*, 355; (e) Kopelman, P. G. *Nature* **2000**, *404*, 635.
2. Watanabe, R.; Kita, M.; Uemura, D. *Tetrahedron Lett.* **2002**, *43*, 6501.
3. (a) Green, H.; Kehinde, O. *Cell* **1973**, *1*, 113; (b) Green, H.; Meuth, M. *Cell* **1974**, *3*, 127.
4. The preadipocyte cell line 3T3-L1, purchased from Human Science Research Resources Bank, Japan Health Sciences Foundation (Osaka, Japan), was cultured in DMEM with 10% FCS in two 96-well plates at 37 °C, 5% CO_2 for 4–7 days (see Ref. 5). After the cells reached 100% confluence, the culture buffer was changed to a differentiation buffer and the samples dissolved in MeOH or water were added. Differentiation buffer was composed of DMEM containing 10% FCS, 1 μM dexamethasone, 0.5 mM IBMX, 90 U/mL penicillin, 90 μg/mL streptomycin, and 10 μg/mL insulin (see Ref. 6). After 7 days, the differentiated 3T3-L1 adipocytes in a 96-well plate were treated with 2% Triton-X 100 for 30 min at room temperature followed by sonication for 1 min. Fat accumulation was determined by measuring liberated triglyceride using a Triglyceride E-test Kit (Wako). To determine the cell viability of differentiated 3T3-L1 adipocytes, another 96-well plate was treated with a Cell Counting Kit-8 Test (Wako), and the absorbance at 450 nm was measured. Values are means ± SD for duplicate samples. (−)-Noradrenaline bitartrate hydrate used as a standard was less effective ($EC_{50} = 42$ μg/mL).
5. (a) Green, H.; Kehinde, O. *Cell* **1976**, *7*, 105; (b) Sekiya, K.; Okuda, H.; Hotta, Y.; Arichi, S. *Phytotherapy Res.* **1987**, *1*, 58; (c) Russell, T. R. *Method Enzymol.* **1981**, *72*, 720.
6. (a) Green, H.; Kehinde, O. *Cell* **1975**, *5*, 19; (b) Rubin, C. S.; Hirsch, A.; Fung, C.; Rosen, O. M. *J. Biol. Chem.* **1978**, *253*, 7570.
7. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.
8. Dinitrophenyl alanyl amide (DAA) derivatives of β-OH-Leu and D-*allo*-Ile were not clearly detected on HPLC analysis due to other contaminants.
9. Miller, R.; Galitsky, N. M.; Duax, W. L.; Langs, D. A.; Pletnev, V. Z.; Ivanov, V. T. *Int. J. Pept. Protein Res.* **1993**, *42*, 539.
10. Feng, Y.; Blunt, J. W.; Cole, A. L. J.; Cannon, J. F.; Robinson, W. T.; Munro, M. H. G. *J. Org. Chem.* **2003**, *68*, 2002.
11. *Dictionary of Natural Products*, 3rd ed.; Chapman & Hall: London, 1997; Vol. 10, p 362.
12. (a) Miller, R.; de Titta, G. T.; Langs, D. A.; Weeks, C. M.; Hauptman, H. A.; Chang, C.-S.; Lee, C.-W.; Thuman, P.; Jones, R. *Science* **1992**, *259*, 1430; (b) Langs, D. A. *Acta Crystallogr. D* **1993**, *49*, 158.
13. Voronkino, T. M.; Koreneva, V. M.; Kursanova, G. V.; Muchak, E. T.; Onoprienko, V. V.; Kolos'ov, M. N. U.S.S.R. Patent N517 198, 1974.
14. Hale, K. J.; Manaviazar, S.; Delisser, M. *Tetrahedron* **1994**, *50*, 9181.
15. Sajiki, H.; Hattori, K.; Hirota, K. *J. Org. Chem.* **1998**, *63*, 7990.
16. (a) Davis, J. S. *J. Peptide Sci.* **2003**, *9*, 471; (b) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
17. Spectroscopic data for synthetic ternatin (**1**): $[\alpha]_D^{24.5}$ −35.5 (*c* 0.40, EtOH); IR ($CHCl_3$) 3426, 3344, 3313, 2960, 2933, 2871, 1636, 1526, 1506, 1466, 1409 cm^{-1}; ^1H NMR (800 MHz, C_6D_6) δ 0.57 (t, $J = 7.4$ Hz, 3H), 0.60 (d, $J = 6.9$ Hz, 3H), 0.75 (d, $J = 7.3$ Hz, 3H), 0.79 (m, 1H), 0.90 (m, 1H), 0.90 (d, $J = 6.9$ Hz, 3H), 0.93 (d, $J = 6.9$ Hz, 3H), 0.97 (d, $J = 6.4$ Hz, 3H), 1.01 (ddd, $J = 13.3$, 9.4, 4.1 Hz, 1H), 1.18 (d, $J = 6.9$ Hz, 3H), 1.25 (d, $J = 6.9$ Hz, 3H), 1.25 (m, 1H), 1.27 (d, $J = 7.3$ Hz, 3H), 1.45 (d,

$J = 6.9$ Hz, 3H), 1.54 (d, $J = 6.4$ Hz, 3H), 1.64 (m, 1H), 1.73–1.84 (m, 2H), 2.12 (m, 1H), 2.23 (m, 1H), 2.36 (m, 1H), 2.60 (s, 3H), 2.68 (s, 3H), 2.79 (s, 3H), 3.30 (s, 3H), 3.98 (dd, $J = 9.4$, 2.1 Hz, 1H), 4.31 (dd, $J = 10.1$, 4.0 Hz, 1H), 4.48 (dd, $J = 6.9$, 3.2 Hz, 1H), 5.17 (dd, 1H), 5.20 (td, $J = 8.0$, 2.0 Hz, 1H), 5.66 (q, $J = 6.9$ Hz, 1H), 5.69 (q, $J = 6.9$ Hz, 1H), 5.89 (d, $J = 2.7$ Hz, 1H), 6.23 (d, $J = 6.4$ Hz, 1H), 7.59 (d, $J = 8.7$ Hz, 1H), 7.95 (d, $J = 9.2$ Hz, 1H); ^{13}C NMR (201 MHz, C_6D_6) δ 174.6, 174.5, 174.2, 172.7, 169.9, 168.8 (2C), 76.0, 59.2, 56.3, 55.1, 52.3, 51.3, 49.8 (2C), 40.5, 37.9, 33.7, 30.7, 30.2, 29.7, 29.4 (2C), 26.7, 26.0, 25.3, 23.9, 23.3, 22.6, 21.3 (2C), 15.9, 14.8, 14.2, 13.6, 13.1, 11.6; HRMS (FAB) calcd for $C_{37}H_{67}N_7O_8Na$ $(M+Na)^+$ 760.4949, found 760.4922.