

Termitomycamides A to E, Fatty Acid Amides Isolated from the Mushroom *Termitomyces titanicus*, Suppress Endoplasmic Reticulum Stress

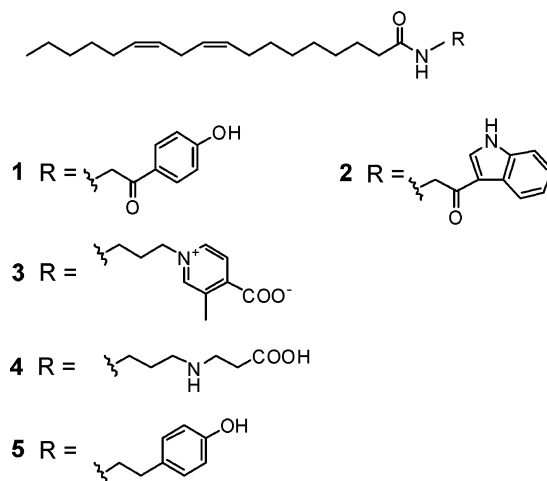
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Received September 13, 2010

ABSTRACT



Five fatty acid amides, termitomycamides A to E (1 to 5), were isolated from the giant edible mushroom *Termitomyces titanicus*. The structures of 1–5 were determined by the interpretation of spectral data and/or synthesis. Compounds 2 and 5 showed protective activity against endoplasmic reticulum stress-dependent cell death.

Endoplasmic reticulum (ER) stress is caused by disturbances in the structure and function of the ER with the accumulation of misfolded proteins and alterations in the calcium homeostasis. The ER response is characterized by changes in

specific proteins, causing translational attenuation, induction of ER chaperones, and degradation of misfolded proteins. In the case of prolonged or aggravated ER stress, cellular signals leading to cell death are activated. ER stress has been suggested to be involved in some human neuronal diseases, such as Parkinson's, Alzheimer's, and prion disease, as well as other disorders.^{1–4} Therefore, the protective activity against ER stress is an important target for the cure or

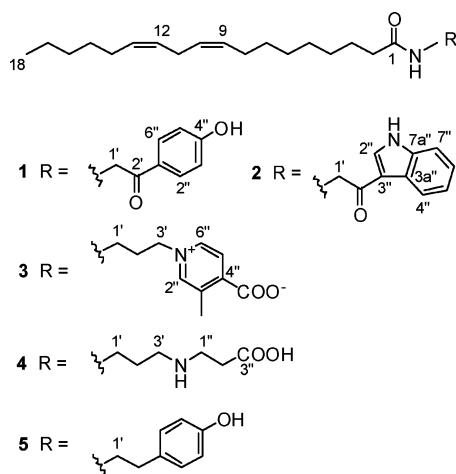
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prevention of these diseases and the demand for new protective substances prompted us to screen the protective activity of the mushroom extracts. We previously reported the protective function of dilinoleoylphosphatidylethanolamine that was isolated from the mushroom *Hericium erinaceum*.⁵ Moreover, we have found new ER stress protective compounds from the mushroom *H. erinaceum*^{6,7} and *Mycophotodanoides aitchisonii*.⁸ During the screening for ER stress protecting effects of the extracts of various mushrooms, we found relatively strong activity in the extract of the mushroom *Termitomyces titanicus* and tried to isolate the active principles from the mushroom. *T. titanicus* with a cap diameter of up to 1 m is the largest edible mushroom in the world according to Guinness Book of Records. It is typified by symbiotic life together with termites. Termites cultivate the mycelia in their nest and fruiting bodies can be seen arising on or near the mounds.⁹ Here, we describe the isolation, structural elucidation, and biological activity of termitomycamides A to E (**1** to **5**).



Powders of the dried fruiting bodies of *T. titanicus* (3.3 kg) were extracted with hexane, EtOAc, and EtOH, successively. Since the EtOAc and EtOH soluble fractions showed the protective activity against ER stress-dependent cell death, the EtOAc soluble fraction was separated using silica gel

column chromatography, followed by HPLC to afford compounds **1**, **2**, and **5** (0.5, 3.4, and 1.0 mg, respectively). On the other hand, the EtOH soluble fraction was partitioned between 1-BuOH and H₂O. The BuOH soluble part was fractionated by gel permeation chromatography, silica gel flash column chromatography, and HPLC to give compounds **3** (3.3 mg) and **4** (1.7 mg).

Termitomycamide A (**1**) was isolated as a colorless oil. Its molecular formula was determined as C₂₆H₃₉NO₃ by HRESIMS [*m/z* 436.2824 [M + Na]⁺ (calcd for C₂₆H₃₉NaNO₃, 436.2828)]. The complete assignment of all the protons and carbons was accomplished by DEPT, HMQC, COSY, and HMBC experiments as shown in Table 1. The DEPT experiment indicated the presence of a methyl,

Table 1. NMR Spectroscopic Data for Termitomycamide A (**1**)^a

position	δ_H (mult, <i>J</i> in Hz)	δ_C	HMBC
1		174.2	
2	2.32 (t, 7.8)	36.6	C-1
3	1.67 (m)	25.6	C-2
4–7, 15	1.23–1.37 (m)	29.1, 29.2, 29.2 29.3, 29.6	
8, 14	2.03 (m), 2.03 (m)	27.2, 27.2	C-9, 10, 12, 13, 16
9, 13	5.36 (m), 5.36 (m)	130.2, 130.5	C-8, 11, 14
10, 12	5.33 (m), 5.33 (m)	127.9, 128.0	C-8, 11, 14
11	2.75 (dd, 6.8, 6.4)	25.6	C-9, 10, 12, 13
16	1.28 (m)	31.5	
17	1.28 (m)	22.6	
18	0.87 (t, 6.9)	14.1	C-16, 17
1'	4.64 (s)	46.0	C-1, 2'
2'		192.2	
1''		126.9	
2'', 6''	7.77 (d, 8.5)	130.5	C-2', 1'', 4''
3'', 5''	6.91 (d, 8.5)	116.0	C-1'', 4''
4''		161.9	

^a CDCl₃, 500 MHz.

13 methylenes, 8 methines, and 4 quaternary carbons. The COSY and HMBC correlations are illustrated in Figure 1. The NMR spectra suggested the presence of a linoleyl moiety including two disubstituted double bonds [δ_H 5.36 (H-9, H-13, 2H), δ_H 5.33 (H-10, H-12, 2H); δ_C 127.9 (C-9), 128.0 (C-10), 130.2 (C-12), 130.5 (C-13), 174.2 (C-1)]. The 2-amino-1-(4-hydroxyphenyl)ethanone moiety was confirmed by the COSY correlations (H-2''/H-3'', H-5''/H-6''), HMBC correlations (H-1'/C-2', H-2''/C-2', H-2''/C-1'', H-2''/C-4'', H-3''/C-1'', H-3''/C-4''), and chemical shifts of H-1' (δ_H 4.64) and C-2' (δ_C 192.2). The connection between the amine and linoleyl moiety was indicated by the HMBC correlation from H-1' to the amide carbonyl at C-1 (δ_C 174.2), the lower chemical shift of H-1' (δ_H 4.64), and the molecular formula of **1**.

Termitomycamide B (**2**) was purified as a colorless oil. Its molecular formula was determined as C₂₈H₄₀N₂O₂ by HRESIMS [*m/z* 459.2990 [M + Na]⁺ (calcd for C₂₈H₄₀NaN₂O₂, 459.2988)]. The ¹H and ¹³C NMR data of the position from 1 to 18, C-1', and C-2' of **2** were very similar to those of **1**. The indole moiety was constructed by the COSY correlations

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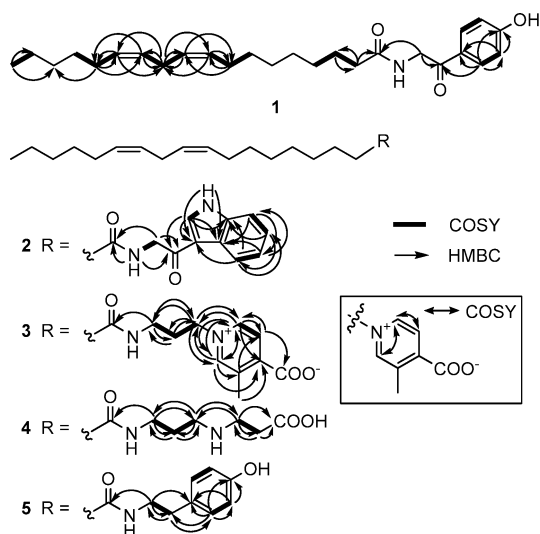


Figure 1. COSY and HMBC correlations of **1**–**5**.

(H-1''/H-2'', H-4''/H-5'', H-6''/H-7''), HMBC correlations (H-1''/C-3'', H-1''/C-3a'', H-2''/C-3'', H-2''/C-3a'', H-4''/C-3'', H-4''/C-6'', H-4''/C-7a'', H-5''/C-3a'', H-5''/C-7'', H-6''/C-4'', H-6''/C-7a'', H-7''/C-3a'', H-7''/C-5''), and the molecular formula of **2**. The structure for **2** was confirmed by its synthesis from linoleic acid and the corresponding amine.

Termitomycamide C (**3**) was isolated as a colorless oil. Its molecular formula was determined as $C_{28}H_{44}N_2O_3$ by HRESIMS [m/z 479.3262 $[M + Na]^+$ (calcd for $C_{28}H_{44}NaN_2O_3$, 479.3250)]. The 1H and ^{13}C NMR data of position 1 to 18 of **3** were similar to those of **1**. The moiety of a 3,4-disubstituted pyridine ring in **3** was suggested by coupling constants and chemical shift: δ_H 8.72 (1H, d, $J = 6.1$ Hz), δ_H 7.84 (1H, d, $J = 6.1$ Hz), and δ_H 8.79 (br s) and the COSY correlations (H-2''/H-6'', H-5''/H-6''). The ^{13}C NMR, DEPT spectra and HMBC correlations (H-2''/C-3'', H-2''/C-4'', H-2''/C-6'', H-5''/C-6'', H-6''/C-2'', H-6''/C-4'', H-6''/C-5'') also indicated the presence of a 3,4-disubstituted pyridine ring. The position of the methyl group at the pyridine was elucidated by HMBC correlations (H-2''/C-3''-Me, H-3''-Me/C2'', H-3''-Me/C-3'', H-3''-Me/C-4''). The position of the carboxyl group in the ring was assigned by the HMBC correlation from H5'' to 4''-COO. The propyl group was determined by the COSY correlations (H-1'/H-2', H-2'/H-3') and HMBC correlations (H-1'/C-2', H-1'/C-3', H-2'/C-1', H-2'/C-3', H-3'/C-1', H-3'/C-2'). The connection between the propyl and the ring was determined by HMBC correlations (H-3'/C-2'', H-3'/C-6'', H-2''/C-3', H-6''/C-3'). The junction between the linoleyl moiety and propylpyridinium group was determined by HMBC correlation from H1' to C-1 (δ_C 176.7). As a result, the structure of **3** was determined as shown.

Termitomycamide D (**4**) was purified as a colorless oil. Its molecular formula was determined as $C_{24}H_{44}N_2O_3$ by HRESIMS [m/z 431.3252 $[M + Na]^+$ (calcd for $C_{24}H_{44}NaN_2O_3$, 431.3250)]. The 1H and ^{13}C NMR data of position 1 to 18 of **4** were very similar to those of **3**. The structure elucidation using NMR was accomplished in the same manner as for **1**. The moiety of the propanoic acid

was determined by the COSY correlation (H-1''/H-2''), HMBC correlations (H-1''/C-2'', H-1''/C-3'', H-2''/C-1'', H-2''/C-3''), and the downfield chemical shift C-3'' (δ_C 177.7). The three-methylene chain was constructed by COSY correlations (H-1'/H-2', H-2'/H-3') and HMBC correlations (H-1'/C-2', H-1'/C-3', H-2'/C-1', H-2'/C-3', H-3'/C-1', H-3'/C-2'). The connection between linoleyl moiety and the other parts (the acid and the three-methylene chain) was determined by HMBC correlations (H-1'/C-1, H-3'/C-1'', H-1''/C-3') and the molecular formula of **4**. As a result, the structure of **4** was determined.

Termitomycamide E (**5**) was isolated as a colorless oil. Its molecular formula was determined as $C_{26}H_{41}NO_2$ by HRESIMS [m/z 422.3034 $[M + Na]^+$ (calcd for $C_{26}H_{41}NaNO_2$, 422.3035)]. The NMR data of **5** were very similar to those of **1**. Comparison of the molecular formula and NMR data of **5** with those of **1** indicates that **5** possesses a methylene instead of a carbonyl of **1**. Although compound **5** already has been reported as an intermediate of organic synthesis,¹² this is the first reported isolation of it from a natural source. The structure was confirmed by synthesis.

In addition, there is no report of the isolation of the corresponding amines in **1** to **4** from nature.

The compounds were subjected to the protective activity assay against ER stress-dependent cell death caused by tunicamycin (TM). ER stress was induced by addition of TM into the culture medium of Neuro2a cells in the presence or absence of each compound. TM is an inhibitor of *N*-linked glycosylation and the formation of *N*-glycosidic protein–carbohydrate linkages.¹¹ It specifically inhibits dolichol pyrophosphate-mediated glycosylation of asparaginyl residues of glycoproteins¹² and induces “ER stress”. Compounds **2** and **5** showed significant protective activity against TM-toxicity dose-dependently (Figure 2). On the other hand, **3**

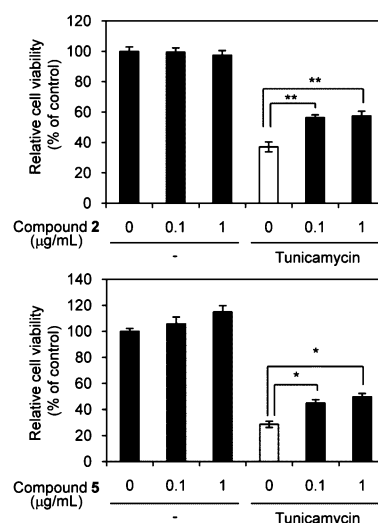


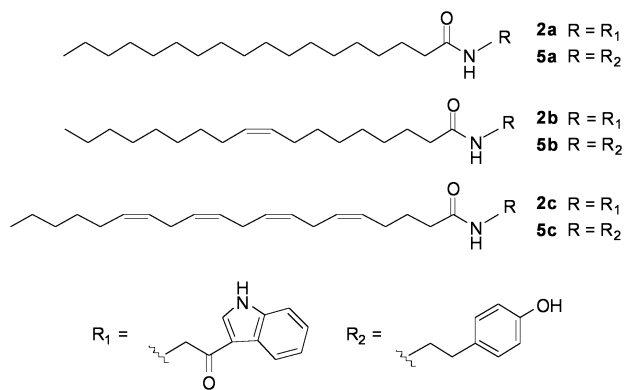
Figure 2. Protective activity of **2** and **5** against ER stress-dependent cell death. The cell viabilities were analyzed by MTT assay, and the values were represented as the mean $\pm$ SEM of the relative percentage of surviving cells compared with the untreated cells ($n = 16$). (*) $p < 0.05$, (**) $p < 0.01$, Tukey–Kramer multiple comparisons tests.

and **4** did not have any activity even at the concentrations up to 1 $\mu\text{g/mL}$ (see Figure S1 in the Supporting Information).

To investigate further the structure–activity relationship on protective activity against ER stress-dependent cell death, analogues of **2** and **5** that have different fatty acid parts (stearyl, oleyl, and arachidonyl) from each other were synthesized (Scheme 1). Although all the amides (**2a–c** and

5a–c) and their precursors (the three acids and the two amines) were examined, all of them showed no significant protective activity against TM-toxicity (data not shown). In addition, linoleic acid also did not exhibit the protective activity similar to the previous report (data not shown).⁵ These results indicated that the linoleyl moiety in **2** and **5** was indispensable for the activity of the compounds.

Scheme 1. The Structures of Compounds (**2a–c** and **5a–c**)



Acknowledgment. We thank V. K. Deo (Shizuoka University) for valuable discussions. This work was partially supported by a grant-in-aid for scientific research on priority areas “Creation of Biologically Functional Molecules” (No. 17035037) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Supporting Information Available: Experimental procedures including bioassay and synthesis, ^1H and ^{13}C NMR spectra of **1–5**, and protecting effect of **3** and **4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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