Pergamon

Tetrahedron 58 (2002) 1103–1105

TETRAHEDRON

Thelephorin A: a new radical scavenger from the mushroom *Thelephora vialis*

Sachiko Tsukamoto,[a] Abdulgafor D. Macabalang,[a] Takamasa Abe,[b] Hiroshi Hirota[b,c] and Tomihisa Ohta[a,*]

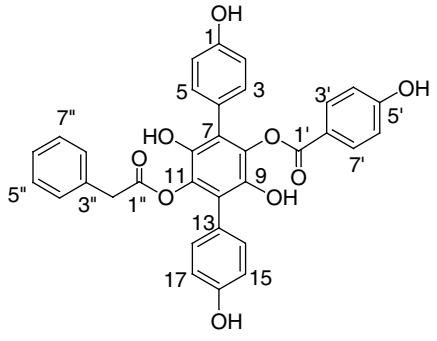
[a]*Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan*
[b]*Genomic Sciences Center (GSC), RIKEN (The Institute of Physical and Chemical Research), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan*
[c]*Graduate School of Integrated Science, Yokohama City University, 1-7-29 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan*

Received 9 November 2001; accepted 7 December 2001

Abstract—A new radical scavenger, thelephorin A, was isolated from the fruiting bodies of the mushroom *Thelephora vialis*. Its structure was elucidated by spectroscopic methods. Thelephorin A showed antioxidative activity.

1. Introduction

Thelephoraceae is a terrestrial fungus family whose species are widely distributed in North America,[1] New Zealand, Australia,[2] and some parts of Japan. Phytochemical investigation of a species belonging to the *Thelephoraceae* family revealed some biologically active compounds which include an antibiotic, phellanic acid,[3] from *Phellon melaleucus* and fungus pigments, xylerythrin,[4] 5-*O*-methyl-xyerythrin,[4] peniophorin,[5] and peniosanguin,[6] from *Peniophora sanguinea*. In our search for naturally occurring, biologically active compounds from the fungus family *Thelephoraceae*, an investigation was undertaken on the chemical constituents of *Thelephora vialis*. This paper describes the isolation, structure elucidation and biological activity of new radical scavenger, thelephorin A (**1**) (Chart 1).



1

Chart 1.

2. Results and discussion

The fruiting body of the mushroom *T. vialis* (0.95 kg, fresh wt) was extracted with MeOH, and the concentrated aqueous residue was extracted with EtOAc. The EtOAc

Table 1. NMR spectral data for thelephorin A (**1**) in CD_3OD

	δ_H	*J* (Hz)	δ_C	HMBC
1			157.9 s	
2, 6	6.73 (2H)	d, 8.7	116.3 d	C-1, C-3, C-4, C-5
3, 5	7.22 (2H)	d, 8.7	132.6 d	C-1, C-2, C-6, C-7
4			125.0 s	
7			124.1 s	
8			135.0 s	
9			142.5 s	
10			123.9 s	
11			135.1 s	
12			142.6 s	
13			124.9 s	
14, 18	7.17 (2H)	d, 8.7	132.7 d	C-10, C-15, C-16, C-17
15, 17	6.82 (2H)	d, 8.7	116.1 d	C-13, C-16
16			158.2 s	
1′			166.2 s	
2′			121.0 s	
3′, 7′	7.70 (2H)	d, 8.7	133.4 d	C-1′, C-2′, C-4′, C-5′, C-6′
4′, 6′	6.78 (2H)	d, 8.7	116.0 d	C-2′, C-3′, C-5′, C-7′
5′			164.1 s	
1″			171.5 s	
2″	3.33 (2H)	s	41.3 t	C-1″, C-3″, C-4″, C-8″
3″			134.4 s	
4″, 8″	6.81 (2H)	d, 8.7	130.2 d	C-2″, C-5″, C-6″, C-7″
5″, 7″	7.02 (2H)	t, 8.7	129.4 d	C-3″, C-5″, C-6″, C-7″, C-8″
6″	7.05 (1H)	t, 8.7	127.9 d	C-8″

Keywords: radical scavenger; mushroom; *Thelephora vialis*.
* Corresponding author. Tel./fax: +81-76-234-4417;
e-mail: ohta@dbs.p.kanazawa-u.ac.jp

0040–4020/02/$ - see front matter
PII: S0040-4020(01)01214-5

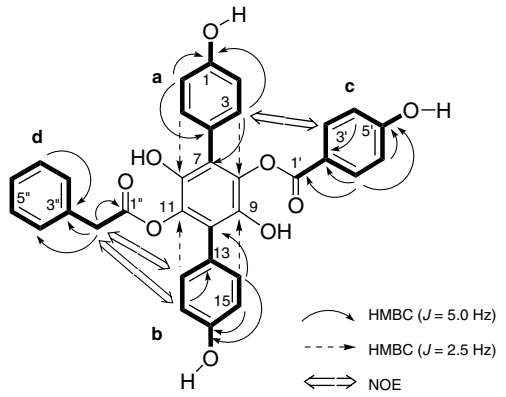


Figure 1. HMBC and NOE correlations observed for thelephorin A (**1**); partial structures **a**–**d** suggested by COSY and HMBC (optimized *J* value: 5 Hz) spectra were drawn in bold line.

layer was successively fractionated by Si gel chromatography ($CHCl_3$/MeOH), ODS chromatography (MeOH/H_2O), and Sephadex LH-20 gel filtration (MeOH) to afford a grayish pigment, thelephorin A (**1**, 33.8 mg, 3.5×10^{-3}%).

Thelephorin A (**1**), a grayish solid, was analyzed for $C_{33}H_{24}O_9$, on the basis of its combined HRFABMS and ^{13}C NMR spectral features (Table 1), which required 22 degrees of unsaturation. The IR spectrum of **1** showed intense absorptions for hydroxy (3425 cm^{-1}), ester (1730, 1700, 1160, and 1100 cm^{-1}), and benzene (1600 cm^{-1}). The ^1H NMR spectrum in CD_3OD showed seven doublets (each 2H, J=8.7 Hz) and two triplet signals (δ 7.02 (2H, J=8.7 Hz) and 7.05 (1H, J=8.7 Hz)) in the low-field region (Table 1), assignable to three 1,4-disubstituted and a mono-substituted phenyl groups, and one singlet signal at δ 3.33 (2H). Acetylation of **1** afforded a pentaacetate, indicating the presence of five hydroxy groups in **1**. Interpretation of the COSY and HMBC (optimized *J* value: 5.0 Hz) spectra led to partial structures **a**–**d** (Fig. 1). Units **a** and **b** were indicated to be *para*-substituted phenyl groups attached to aromatic carbons at δ 123.9 (s) and 124.1 (s). Unit **c** was a *para*-substituted benzoyl group, suggested by HMBC correlation between aromatic hydrogens (H-3′ and H-7′) and a carbonyl carbon at δ 166.2 (C-1′). The presence of hydroxy groups at C-1, C-16, and C-5′ in units **a**–**c** was suggested by the chemical shifts of δ 157.9 (C-1), 158.2 (C-16), and 164.1 (C-5′). Unit **d** was a phenylacetoxyl group; HMBC correlations were observed between a singlet methylene signal at δ 3.33 and an ester carbon at δ 171.5 (C-1″) and mono-substituted aromatic carbons at δ 130.2 (C-4″ and C-8″) and 134.4 (C-3″). The HMBC spectrum measured at an optimized *J* value of 2.5 Hz exhibited four-bond correlations, δ 7.22 (H-3 and H-5)/δ 135.0 (C-8) and 142.6 (C-12); δ 7.17 (H-14 and H-18)/δ 142.5 (C-9) and 135.1 (C-11) (Fig. 1). The molecular formula of **1** suggested that the remaining four quaternary carbons formed a six-substituted aromatic ring together with the carbons at δ 124.1 (C-7) and 123.9 (C-10), that are linked to units **a** and **b**, respectively. The remaining four carbons were linked to the benzoyl group (unit **c**), phenylacetoxyl group (unit **d**), and two hydroxyl groups which were deduced from the remaining H_2O_2 unit. To determine the positions of the hydroxy substituents, the ^{13}C NMR spectrum in CD_3OH was measured and the low-field shifts from those in CD_3OD were observed for signals at C-1, C-9, C-12, C-16, and C-5′ (Fig. 2). Therefore, the hydroxy groups were bonded to carbons, C-9 and C-12, and the presence of three hydroxy groups at C-1, C-16, and C-5′ was confirmed. The NOESY spectrum (mixing time: 800 ms) of **1** exhibited cross peaks for H-3/H-3′ (unit **a**/unit **c**) and H-2″/H-14 and H-15 (unit **b**/unit **d**), which substantiated that units **c** and **d** were bonded to C-8 and C-11, respectively. Thus, the structure of thelephorin A (**1**) was established as shown.

Free radical scavenging activity of thelephorin A (**1**) was evaluated against the stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH·). Antioxidant activity was defined as the amount of antioxidant necessary to decrease the initial DPPH· concentration by 50% (EC_{50}, antioxidant (mol/L)/DPPH· (mol/L)). The results obtained showed that **1** is a potent antioxidant compound with EC_{50} of 0.028, which is 10 times as active as ascorbic acid (EC_{50}=0.27).

Derivatives of 1,2,4,5-tetrahydroxy-3,6-di(*p*-hydroxyphenyl)benzene have been reported from *Paxillus atrotomentosus*,[7] *Paxillus panuoides*,[8] and *Polyozellus multiplex*,[9] some of which were also reported as potent antioxidants. Polyphenols scavenge superoxide radicals and hydroxy radicals, reduce lipid peroxyl radicals, and inhibit lipid peroxidation. Free radicals cause peroxidative disintegration of cells implicated in various pathological processes and especially are involved in pathogenesis of diseases such as myocardial and cerebral ischemia, cancer-initiation, and the aging process. Thelephorin A (**1**) can be a potential agent against these diseases.[10]

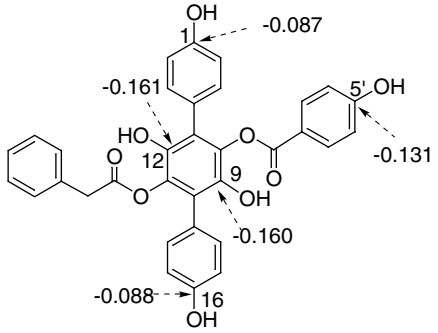


Figure 2. Deuterium isotope shifts ($\delta_{CD3OD}-\delta_{CD3OH}$) observed for ^{13}C NMR of the thelephorin A (**1**); values more than 0.08 ppm were shown.

3. Experimental

3.1. General

UV spectrum was measured on a SHIMADZU UV-1600 UV–Visible spectrophotometer. IR spectrum was recorded on a SHIMADZU IR-460 infrared spectrophotometer. NMR spectra were recorded on a Bruker Avance600 NMR spectrometer in CD_3OD or CD_3OH. All chemical shifts were reported with respect to TMS. Mass spectra were measured on a JEOL SX-102 or a JEOL GCmate mass spectrometer.

3.2. Extraction and isolation

The mushroom *T. vialis* was collected in Yamagata Prefecture, Japan, in September, 2000. The MeOH extract of the fruiting bodies (0.95 kg, fresh wt) was concentrated and partitioned between water and EtOAc. The EtOAc layer (5.37 g) was subjected to Si gel chromatography with $CHCl_3$/MeOH. The fraction (761.4 mg) eluted with $CHCl_3$/MeOH (8:2) was purified by ODS chromatography with 50% MeOH/H_2O followed by Sephadex LH-20 gel filtration with MeOH to afford thelephorin A (**1**, 33.8 mg, 3.5×10^{-3}%).

3.2.1. Thelephorin A (1). UV (MeOH) λ_{max} (log ϵ) 207.5 (4.8), 262.0 (4.6), 405.5 nm (2.3); IR (film) ν_{max} 3425, 1730, 1700, 1600, 1510, 1450, 1260, 1160, 1100 cm^{-1}. ^1H and ^{13}C NMR (CD_3OD) see Table 1. FABMS (positive, 3-nitrobenzyl alcohol) *m/z* 565 $[M+H]^+$; HRFABMS (positive, PEG600 in 3-nitrobenzyl alcohol) *m/z* 565.1499 ($C_{33}H_{25}O_9$, Δ 0 mmu).

3.2.2. Acetylation of thelephorin A. A solution of thelephorin A (**1**, 3.0 mg) in acetic anhydride (0.3 mL) was added to pyridine (0.3 mL). The reaction mixture was left overnight at room temperature. 3 mL of water was added to the reaction mixture, and evaporation of the reagents and water gave a white amorphous solid product (4.0 mg): ^1H NMR ($CDCl_3$) δ 1.96 (3H, s), 1.97 (3H, s), 2.24 (3H, s), 2.32 (3H, s), 2.33 (3H, s), 3.34 (2H, s), 6.83 (2H, d, *J*= 6.8 Hz), 7.01 (2H, t, *J*=6.8 Hz), 7.04 (2H, d, *J*=6.8 Hz), 7.08 (2H, d, *J*=6.8 Hz), 7.09 (1H, t, *J*=6.8 Hz), 7.10 (2H, d, *J*=6.8 Hz), 7.30 (2H, d, *J*=6.8 Hz), 7.33 (2H, d, *J*= 6.8 Hz), 7.82 (2H, d, *J*=6.8 Hz); EIMS *m/z* 774 (M^+); HREIMS *m/z* 774.1945 ($C_{43}H_{34}O_{14}$, Δ −0.3 mmu).

3.3. Free-radical scavenging assay (DPPH test)

Assay of the antioxidant activity of thelephorin A (**1**) was performed using the stable free radical, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·).[11] DPPH· has an absorption maximum at 515 nm, which disappears upon reduction by an antiradical compound. An aliquot (0.1 mL) of the MeOH solution containing different amounts of **1** was added to 3.9 mL of 6.0×10^{-5} M DPPH· in MeOH. Ascorbic acid was used as a positive control. Absorbance at 515 nm was measured until the reaction reached a steady state. Antioxidant activity was defined as the amount of antioxidant necessary to decrease the initial DPPH· concentration by 50% (EC_{50}, antioxidant (mol/L)/DPPH· (mol/L)).

Acknowledgements

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan, the Hayashi Memorial Foundation for Female Natural Scientists, the Fugaku Trust for Medicinal Research, and the Naito Foundation.

References

1. McKnight, K. H.; McKnight, V. B. *A Field Guide to Mushroom, North America*; Houghton Mifflin: Boston, 1987; pp 76–78.
2. Cunningham, G. H. *Bull. NZ Dept. Sci. Ind. Res.* **1963**, *145*, 1–359.
3. Stadler, M.; Anke, T.; Dasenbrock, J.; Steglich, W. *Z. Naturforsch.* **1993**, *48*, 545–549.
4. Gripenberg, J.; Martikkala, J. *Acta Chem. Scand.* **1969**, *20*, 2583–2588.
5. Gripenberg, J.; Martikkala, J. *Acta Chem. Scand.* **1970**, *24*, 3444–3448.
6. Gripenberg, J. *Acta Chem. Scand.* **1971**, *25*, 2999–3005.
7. Holzapfel, M.; Kilpert, C.; Steglich, W. *Liebigs Ann. Chem.* **1989**, 797–801.
8. Yun, B.-S.; Lee, I.-K.; Kim, J.-P.; Yoo, I.-D. *J. Antibiotics* **2000**, *53*, 711–713.
9. Lee, H.-J.; Rhee, I.-K.; Lee, K.-B.; Yoo, I.-D.; Song, K.-S. *J. Antibiotics* **2000**, *53*, 714–719.
10. Thelephorin A (**1**) was not cytotoxic against HeLa cells at 50 μg/mL.
11. Brand-Williams, W.; Cuvelier, M. E.; Berset, C. *Lebensm.-Wiss. u.-Technol.* **1995**, *28*, 25–30.