Fitoterapia 129 (2018) 108–113

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Three bisabolane-type sesquiterpenes from edible mushroom *Pleurotus eryngii*

Takashi Kikuchi[a,*], Kazutaka Kitaura[a], Ayaka Katsumoto[a], Jie Zhang[b], Takeshi Yamada[a], Reiko Tanaka[a]

[a] Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan
[b] School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing 211198, PR China

ARTICLE INFO

Keywords:
Bisabolane-type
Sesquiterpene
Pleurotus eryngii
Inhibitory activity on nitric oxide production

ABSTRACT

Three bisabolane-type sesquiterpenes (**1**–**3**) were isolated from the fruiting bodies of king trumpet mushrooms (*Pleurotus eryngii*), together with a known compound (**4**). All isolated compounds were evaluated for their inhibitory effects on nitric oxide (NO) production. Among these, **2** exhibited a moderate inhibitory effect on NO production with an IC_{50} of 90.9 μM.

1. Introduction

The edible mushroom *Pleurotus eryngii* (Pleurotaceae) is native to the Mediterranean Basin, Central and Southern Europe, and Central and Western Asia [1]. It was found to contain the following bioactive compounds: eryngiolide A, exhibiting cytotoxicity to tumor cells [2], pleurone, showing an inhibitory effect on human neutrophil elastase [3], polysaccharides, having an inhibitory effect on lipid accumulation [4, 5] and showing antitumor activity [6], a polypeptide showing antioxidant, antitumor, and immunostimulatory activities [7], and a protein exhibiting cytotoxicity to tumor cells [8]. In our continuing search for bioactive compounds from *P. eryngii*, we recently reported the isolation of ergostane-type steroids, including eringiacetals A and B and pleurocins A and B, with evaluations of their inhibitory effects on nitric oxide (NO) production and human recombinant aromatase [9–11]. In this paper, we describe the isolation and structural elucidation of three new bisabolane-type sesquiterpenes, and the evaluation of their inhibitory effects on NO production.

2. Experimental

2.1. General experimental procedures

Chemicals and reagents were purchased as follows: fetal bovine serum (FBS) from *Invitrogen Co.* (Carlsbad, CA, USA); 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) from Sigma-Aldrich Japan Co. (Tokyo, Japan); Dulbecco's modified Eagle's medium (DMEM), antibiotics, and lipopolysaccharide (LPS) from *Escherichia coli* O157, from Nacalai Tesque, Inc. (Kyoto, Japan); sulfanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); and N^G-monomethyl-L-arginine acetate (L-NMMA) from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). All other chemicals and reagents were of analytical grade. Physical data were obtained using following instruments: a JASCO DIP-1000 digital polarimeter for optical rotations; a Jasco FT/IR-680 Plus for IR spectra; an Agilent-NMR-vnmrs600 for ^1H and ^{13}C NMR spectra (^1H: 600 MHz; ^{13}C: 150 MHz) in $CDCl_3$ with tetramethylsilane as the internal standard; and a JEOL JMS-700 for FAB mass spectrometry. Column chromatography was carried out with silica gel (70–230 mesh, Merck, Darmstadt, Germany) and silica gel 60 (230–400 mesh, Nacalai Tesque, Inc., Kyoto, Japan). HPLC was performed using the following systems; system I: MeOH/H_2O (45:55), system II: MeOH/H_2O (55:45), system III: MeOH/H_2O (60:40), and system IV: MeOH/H_2O (80:20), with a Cosmosil $5C_{18}$-MS-II column (25 cm × 20 mm i.d.) (Nacalai Tesque, Inc.) 4.0 mL/min, 35 °C.

2.2. Material

Fruiting bodies of *P. eryngii*, produced in Kagawa, Japan, were purchased from HOKUTO Corp. in 2014.

2.3. Extraction and isolation

The fruiting bodies of *P. eryngii* (dry weight 13 kg) were extracted with MeOH under reflux (3 days, 4 times). The MeOH extract (1920 g) was partitioned between AcOEt and H_2O. The AcOEt-soluble fraction

* Corresponding author.
E-mail address: t.kikuchi@gly.oups.ac.jp (T. Kikuchi).

https://doi.org/10.1016/j.fitote.2018.06.021
Received 21 May 2018; Received in revised form 21 June 2018; Accepted 22 June 2018
Available online 26 June 2018
0367-326X/

(385 g) was subjected to SiO_2 column chromatography (CC) [SiO_2 (3.5 kg); hexane/AcOEt (5:1, 1:1, and 0:1), and AcOEt/MeOH (10:1, and 0:1) in increasing order of polarity] resulting in fourteen fractions (Fr. A–N).

Fr. F (33 g), which was eluted with hexane/AcOEt (1:1), was fractionated into 16 fractions, F1 to F16, by SiO_2 CC; Fr. F5 (33 g) was then eluted with hexane:AcOEt 5:1, and rechromatographed by SiO_2 CC to yield 18 fractions, F5–1 to F5–18. Preparative HPLC (system II) of Fr. F5–6 (56.04 mg), eluted with hexane:AcOEt 1:1, gave **1** (3.65 mg; t_R 51.6 min). SiO_2 CC of Fr. F6 (5 g), eluted with hexane:AcOEt 5:1, gave 35 fractions, F6–1 to F6–35. Among these, F6–11 (183.03 mg), eluted with AcOEt, gave **2** (2.63 mg; t_R 39.9 min), **1** (1.81 mg; t_R 46.3 min), and **3** (14.00 mg; t_R 52.7 min) by HPLC (system III).

Fr. G (1 g), eluted with hexane:AcOEt 1:1, was subjected to SiO_2 CC to yield 14 fractions, G1 to G14. Preparative HPLC (system I) of G6 gave **4** (2.81 mg; t_R 90.8 min).

Fr. H (22 g), eluted with hexane:AcOEt 1:1, was subjected to SiO_2 CC to yield 8 fractions, H1 to H8; H2 (17 g), eluted with hexane:AcOEt 1:1, was then rechromatographed by SiO_2 CC to yield 38 fractions. Among these, H2–10 (49.92 mg) and H2–11 (51.80 mg), eluted with AcOEt, gave **1** (1.05 mg and 1.19 mg, respectively; t_R 16.7 min) by HPLC (system IV).

2.3.1. Compound **1**

Amorphous solid; $[\alpha]_D^{20}$ + 92.8 (*c* 0.34, EtOH); IR (KBr) ν_{max} 3446, 2960, 1717, 1456, 1368, 1153, 1101, 1041, 1016 cm^{-1}; for ^1H and ^{13}C NMR spectra, see Table 1; FABMS *m/z* 309 $[M + Na]^+$; HRFABMS *m/z* 309.1676 (calcd for $C_{15}H_{26}O_5Na$, 309.1678).

2.3.2. Compound **2**

Amorphous solid; $[\alpha]_D^{20}$ + 39.7 (*c* 0.046, EtOH); IR (KBr) ν_{max} 3419, 2360, 2342, 1456, 2342, 1456, 1382, 1040 cm^{-1}; for ^1H and ^{13}C NMR spectra, see Table 1; FABMS *m/z* 295 $[M + Na]^+$; HRFABMS *m/z* 295.1883 (calcd for $C_{15}H_{28}O_4Na$, 295.1885).

2.3.3. Compound **3**

Amorphous solid; $[\alpha]_D^{20}$ + 182.6 (c 0.20, EtOH); IR (KBr) ν_{max} 3428, 2951, 2865, 1426, 1369, 1259, 1115, 1092 cm^{-1}; for ^1H and ^{13}C NMR spectra, see Table 1; FABMS *m/z* 293 $[M + Na]^+$; HRFABMS *m/z* 293.1730 (calcd for $C_{15}H_{26}O_4Na$, 293.1729).

2.4. Preparation of the (S)-and (R)-MTPA Esters from **1, 2,** and **3**

To a solution of **1** (3.18 mg) in pyridine was added (−)-MTPA-Cl (100 mg). The mixture was stirred at r.t. overnight, poured into H_2O and extracted with CH_2Cl_2. The organic layer was evaporated *in vacuo* to give a crude product. This was subjected to HPLC, which yielded **1a** (3.27 mg), the (*S*)-MTPA ester of **1**. Compound **1b** (2.83 mg), the (*R*)-MTPA ester of **1**, was prepared from **1** (2.41 mg); the (*S*)-MTPA ester of **2** (**2a**, 2.33 mg), and (*R*)-MTPA ester of **2** (**2b**, 4.48 mg) were prepared from **2** (2.38 mg and 2.67 mg, respectively); the (*S*)-MTPA ester of **3** (**3a**, 6.00 mg) and (*R*)-MTPA ester of **3** (**3b**, 5.00 mg) were prepared from **3** (2.41 mg and 2.77 mg, respectively) by a similar method to that described above.

2.4.1. (S)-MTPA ester of compound **1** (**1a**)

^1H NMR (600 MHz, $CDCl_3$) δ 7.63 (2H, brd, *J* = 7.9 Hz, Ar), 7.39 (overlapped, Ar), 7.37 (overlapped, Ar), 5.11 (1H, d, *J* = 8.5 Hz, H-7), 4.55 (1H, t, *J* = 8.5 Hz, H-7a), 4.48 (1H, s, 2-OH), 3.61 (3H, s, OMe), 2.78 (1H, dd, *J* = 17.9, 7.3 Hz, H-9B), 2.53 (1H, m, H-3a), 2.40 (1H, dd, *J* = 17.9, 6.2 Hz, H-9A), 2.20 (1H, m, H-3), 2.18 (1H, m, H-10), 2.15 (1H, m, H-4α), 1.72 (1H, ddd, *J* = 14.0, 4.4, 2.0 Hz, H-5α), 1.64 (1H, m, H-4β), 1.58 (1H, m, H-5β),1.09 (3H, s, Me-14), 0.94 (3H, d, *J* = 6.8 Hz, H-11), 0.897 (3H, d, *J* = 6.5 Hz, Me-13), 0.840 (3H, d, *J* = 6.8 Hz, Me-12) ppm; HRFABMS *m/z* 525.2068 (calcd for $C_{25}H_{33}F_3O_7Na$, 525.2076).

2.4.2. (R)-MTPA ester of compound **1** (**1b**)

^1H NMR (600 MHz, $CDCl_3$) δ 7.59 (2H, brd, *J* = 6.7 Hz, Ar), 7.40 (overlapped, Ar), 7.38 (overlapped, Ar), 5.09 (1H, d, *J* = 8.5 Hz, H-7), 4.49 (1H, t, *J* = 8.5 Hz, H-7a), 4.45 (1H, s, 2-OH), 3.47 (3H, s, OMe), 2.80 (1H, dd, *J* = 18.2, 7.3 Hz, H-9B), 2.52 (1H, m, H-3a), 2.43 (1H, dd, *J* = 18.2, 6.4 Hz, H-9A), 2.20 (1H, dq, *J* = 16.7, 6.4 Hz, H-3), 2.18 (1H, m, H-10), 2.16 (1H, m, H-4α), 1.76 (1H, m, H-5α), 1.64 (1H, m, H-4β), 1.60 (1H, m, H-5β), 1.23 (3H, s, Me-14), 0.96 (3H, d, *J* = 6.7 Hz, Me-11), 0.891 (3H, d, *J* = 6.5 Hz, Me-13), 0.888 (3H, d, *J* = 6.7 Hz, Me-12) ppm; HRFABMS *m/z* 525.2075 (calcd for $C_{25}H_{33}F_3O_7Na$, 525.2076).

2.4.3. bis-(S)-MTPA ester of compound **2** (**2a**)

^1H NMR (600 MHz, $CDCl_3$) δ 7.65 (2H, m. Ar), 7.55 (2H, m, Ar), 7.41 (overlapped, Ar), 7.35 (overlapped, Ar), 7.34 (overlapped, Ar), 5.30 (1H, dt, *J* = 9.4, 3.6 Hz, H-8), 4.90 (1H, d, *J* = 8.5 Hz, H-7), 4.18 (1H, dd, *J* = 8.8, 4.4 Hz, H-2), 3.92 (1H, t, *J* = 7.9 Hz, H-7a), 3.550

Table 1
NMR spectroscopic data (^1H: 600 MHz; ^{13}C: 150 MHz) for compound **1–3** in $CDCl_3$.

	1				**2**				**3**			
position	δ_H		δ_C		δ_H		δ_C		δ_H		δ_C	
2			105.3	s	3.97	(1H, dd, 8.2, 5.0)	83.4	d	4.42	(1H, dd, 3.2, 2.3)	83.6	d
3	2.13[a]		41.4	d	2.20	(1H, m)	37.2	d			147.6	s
3a	2.40[a]		42.2	d	2.03	(1H, m)	44.2	d	2.89	(1H, br s)	42.6	d
4	α 2.12[a]		18.4	t	α 1.96	(1H, m)	19.9	t	α 2.10	(1H, m)	18.1	t
	β 1.56	(1H, m)			β 1.48[a]				β 1.78	(1H, m)		
5	α 1.69	(1H, m)	32.4	t	α 1.65	(1H, m)	32.1	t	α 1.55	(1H, m)	31.7	t
	β 1.48	(1H, td, 14.0, 4.7)			β 1.45[a]				β 1.46	(1H, m)		
6			72.0	s			72.0	s			72.4	s
7	3.38	(1H, d, 8.3)	77.7	d	3.35	(1H, d, 7.4)	75.4	d	3.16	(1H, d, 8.2)	75.0	d
7a	4.28	(1H, t, 8.3)	83.8	d	4.06	(1H, t, 7.4)	81.9	d	4.22	(1H, t-like, 8.2)	82.9	d
8			208.5	s	3.74	(1H, ddd, 10.0, 5.0, 2.4)	70.6	d	3.74	(1H, dt, 10.3, 3.2)	71.9	d
9	A 2.43	(1H, dd, 17.3, 7.1)	43.9	t	A 1.35	(1H, m)	42.5	t	A 1.20	(1H, ddd, 12.7, 10.3, 3.0)	40.1	t
	B 2.64	(1H, dd, 17.3, 6.5)			B 1.47[a]				B 1.50	(1H, m)		
10	2.23	(1H, m)	24.3	d	1.83	(1H, m)	24.6	d	1.85	(1H, m)	24.5	d
11	0.96	(3H, d, 6.7)	22.6	q	0.92	(1H, d, 6.5)	21.5	q	0.90	(3H, d, 6.5)	21.6	q
12	0.94	(3H, d, 6.7)	22.5	q	0.96	(1H, d, 6.5)	24.0	q	0.95	(3H, d, 6.5)	23.8	q
13	0.88	(3H, d, 6.4)	11.0	q	1.09	(3H, d, 7.0)	13.4	q	A 4.99	(1H, brs)	105.4	t
									B 5.03	(1H, brs)		
14	1.32	(3H, s)	27.0	q	1.26	(3H, s)	26.7	q	1.24	(3H, s)	26.9	q
2-OH	4.20	(1H, s)										

[a] Overlapped with other signals.