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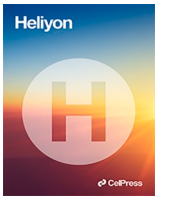

Research article

Inaoside A: New antioxidant phenolic compound from the edible mushroom *Laetiporus cremeiporus*

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A R T I C L E I N F O

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A B S T R A C T

Laetiporus cremeiporus is the edible mushroom distributed in East Asia. It has been reported that an extract of *L. cremeiporus* exhibited DPPH radical scavenging activity. The aim of this study is to identify the antioxidant compound from *L. cremeiporus*. Guided by DPPH radical scavenging activity, a new antioxidant phenolic compound inaoside A (**1**) and three well-known bioactive compounds 5′-*S*-methyl-5′-thioadenosine (MTA, **2**), nicotinamide (**3**), and adenosine (**4**) were isolated from *L. cremeiporus*. An antioxidant compound was isolated from *L. cremeiporus* for the first time. This is the first report of the isolation of **1**, **2**, and **4** from *L. cremeiporus*. The structures were determined by one- and two-dimensional NMR spectroscopic analysis and chemical derivatization. The antioxidant activities of extracts, fractions, and isolated compounds were evaluated by a DPPH radical scavenging assay. Compound **1** exhibited significant DPPH radical scavenging activity (80 % inhibition at 100 μg/mL, IC_{50} 79.9 μM, trolox equivalent antioxidant capacity (TEAC) = 0.36).

1. Introduction

The edible mushroom genus *Laetiporus* belongs to the family Polyporaceae [1]. *Laetiporus* is known to be abundant sources of bioactive compounds [2]. Some cytotoxic [3,4] and anti-inflammatory compounds [5,6] from *Laetiporus* have been reported. Additionally, various secondary metabolites have been isolated from *Laetiporus*, including the brassinosteroid analogues [6], 3,4-secolanostane-type triterpenes [7], sesquiterpenoids [4], egonol glucoside [8], and (±)-laetirobin [9]. Extracts of *Laetiporus* reportedly exhibited several bioactivities, including antioxidant [10,11], antimicrobial [12], antithrombin [13], and dopamine D2 receptor agonistic activities [14]. Well-known antioxidant compounds such as (+)-catechin, gallic acid, and quercetin have been isolated from *Laetiporus sulphureus* [15]. However, in the majority of studies on antioxidant activity of *Laetiporus*, isolation and structure determination of

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antioxidant compounds have not been achieved. To date, there has been only one report on the biological function of the species *Laetiporus cremeiporus*, which is distributed in East Asia [16]. Li and co-workers revealed that an extract of *L. cremeiporus* exhibited DPPH radical scavenging activity. By contrast, there have been no reports on the isolation of antioxidant compounds from *L. cremeiporus*.

In this study, guided by DPPH radical scavenging assay, we searched for antioxidant compounds from the methanolic extract of *L. cremeiporus*. A new antioxidant phenolic compound and three well-known bioactive compounds were isolated from *L. cremeiporus*. We report herein on the isolation, structure determination, and bioactivities of these compounds.

2. Material and methods

2.1. General methods

One- and two-dimensional NMR data were obtained with a Bruker AVANCE NEO 400 (400 MHz) spectrometer for ^{1}H NMR and a Bruker AVANCE NEO 400 (100 MHz) spectrometer for ^{13}C NMR using Shigemi 5 mm φ Micro Bottom Tubes (Cat. No. SP-502). Chemical shifts were indicated in parts per million (ppm) with coupling constants are shown in hertz (Hz) relative to the residual solvent peaks at δ_H 2.50 and δ_C 39.5 in DMSO-d_6, and. The abbreviations s, d, dd, t, q, br s and m stand for the resonance multiplicity singlet, doublet, doublet of doublets, triplet, quartet, broad singlet and multiplet, respectively. The IR spectra were recorded on a JASCO FT/IR 480 Plus instrument by attenuated total reflection (ZnSe-ATR) and is shown in wavenumbers (cm^{-1}). High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-T 100LP AccuTOF LC-Express spectrometer (Direct Analysis in Real Time, DART). LC-ESI-MS analysis was performed using a Shimadzu LCMS-8050 spectrometer equipped with the Nexera LC-40D HPLC system. Optical rotations were recorded on a JASCO DIP-1000 polarimeter with the sodium D line. Nacalai Silica gel 60 F254 60−230 mesh and Nacalai Tesque ODS silica gel Cosmosil 140C18-OPN were used for column chromatography. Chemicals and solvents were purchased commercially. Solvents for reverse-phase HPLC were purchased as HPLC grade.

2.2. Mushroom material

Fruiting bodies of *L. cremeiporus* were collected at the Ina campus of Shinshu University (Kami-ina, Japan). Fungal identification was made based on the DNA sequencing of the internal transcribed spacer (ITS) region within the nuclear ribosomal RNA gene operon (DDBJ accession number: LC770055) and a BLAST search following the procedure by Ref. [17].

2.3. Extraction and isolation

The fresh fruiting bodies of *L. cremeiporus* (1.3 kg) were extracted with MeOH (7.2 L) at room temperature for 13 days. The MeOH extract was filtered and then the filtrate was concentrated. The residue was partitioned between water and EtOAc (1.0 L each, four times). The EtOAc layer was concentrated. The residue was partitioned between 90 % aqueous MeOH and hexane (1.0 L each, four

Table 1
NMR data for inaoside A (**1**) in DMSO-d_6.

Atom No.	^{13}C NMR[a)] δ [ppm], multiplicity	^{1}H NMR[b, c)] δ [ppm], multiplicity, *J* [Hz]	HMBC ^{1}H → ^{13}C	NOESY ^{1}H → ^{1}H	ROESY ^{1}H → ^{1}H
1	137.7 s				
2	121.3 d	6.72 s	C-1, 3, 4, 6, 1′	H-1′, 1″	H-1′, 2′, 1″, 2″
3	118.9 s				
4	150.4 s				
5	101.0 d	6.44 s	C-1, 3, 4, 6	H-6′, 4-OH	H-6′, 4-OH
6	149.1 s				
1′	27.3 t	3.10 d (7.2)	C-2, 3, 4, 2′,3′	H-2, 5′	
2′	123.2 d	5.22 m	C-4′, 5′	H-2, 1′, 4′	H-2, 4′
3′	130.8 s				
4′	25.5 q	1.67 d (0.8)	C-2′, 3′, 5′	H-2′	
5′	17.6 q	1.65 d (0.8)	C-2′, 3′, 4′	H-1′	
6′	55.8 q	3.68 s	C-6	H-5	H-5
1″	102.5 d	5.29 d (4.3)	C-1, 2″, 3″, 4″	H-2, 6′, 2″, 3″	H-2, 2″
2″	71.7 d	3.96 m		H-1″, 5″	H-1″
3″	69.5 d	3.85 m		H-1″, 5″	H-5″, 3″-OH, 5″-OH
4″	86.1 d	3.98 m	C-3″	H-5″	H-5″, 3″-OH, 5″-OH
5″	61.6 t	3.42 dd (5.3, 5.3)	C-3′, 4″	H-3″, 4″, 5″-OH	H-4″, 5″-OH
4-OH		8.98 br s			H-5
2″-OH		4.36 d (8.9)			
3″-OH		4.62 d (6.4)			H-3″, 4″
5″-OH		4.72 t (5.3)	C-4″, 5″	H-5″	H-3″, 4″, 5″

a) ^{13}C NMR spectrum was measured at 100 MHz. Multiplicity was besed on multiplicity-edited HSQC.
b) ^{1}H NMR spectrum was measured at 400 MHz.
c) Coupling constants (Hz) are in parentheses.

times). The 90 % aqueous MeOH layer was concentrated and then the residue partitioned between 60 % aqueous MeOH and CH_2Cl_2 (1.0 L each, four times). The CH_2Cl_2 layer was concentrated to obtain 1.6 g of the CH_2Cl_2 layer. 812.2 mg of the residue out of 1.6 g was fractionated by silica gel column chromatography using 100 g of silica gel (CH_2Cl_2/MeOH = 49/1 → 19/1 → 9/1 → 4/1 → 2/1 → 1/1, 300 mL each) to afford six fractions (Fr. 1 to 6). Fr. 3 (67.2 mg) was fractionated by ODS silica gel column chromatography using 3.0 g of Cosmosil 140C18-OPN (20, 40, 70, and 100 % aqueous MeOH, 18 mL each) to afford four fractions (Fr. 3-1 to 4). Fr. 3-2 (5.4 mg) was finally purified by reverse-phase HPLC (Develosil ODS-HG-5, φ 20 × 250 mm, 23 % aqueous acetonitrile, 5 mL/min, 25 °C, 215 nm, 254 nm) to afford inaoside A (**1**) (1.3 mg, 2.0×10^{-4} %, t_R = 79.9 min). In addition, **1** was contained in Fr. 4 other than Fr. 3, isolation of **1** from Fr. 4 was also carried out. Fr. 4 (73.4 mg) was fractionated by ODS silica gel column chromatography using 3.0 g of Cosmosil 140C18-OPN (20, 40, 70, and 100 % aqueous MeOH, 18 mL each) to give four fractions (Fr. 4-1 to 4). Fr. 4-2 (5.4 mg) was purified by reverse-phase HPLC (Develosil ODS-HG-5, φ 20 × 250 mm, 23 % aqueous acetonitrile, 5 mL/min, 25 °C, 215 nm, 254 nm) to afford **1** (1.1 mg, 1.7×10^{-4} %, t_R = 80.4 min). Fr. 3-1 (8.3 mg) was purified by reverse-phase HPLC (Develosil ODS-HG-5, φ 20 × 250 mm, 30 % aqueous MeOH, 5 mL/min, 25 °C, 215 nm, 254 nm) to afford 5′-*S*-methyl-5′-thioadenosine (MTA, **2**) (0.5 mg, 7.6×10^{-5} %, t_R = 36.8 min) and Fr. 3-1-2. Fr. 3-1-2 (4.4 mg) was finally purified by reverse-phase HPLC {Develosil ODS-HG-5, φ 20 × 250 mm, 10–40 % (linear gradient for 90 min) aqueous MeOH, 5 mL/min, 25 °C, 215 nm, 254 nm} to give nicotinamide (**3**) (0.5 mg, 7.6×10^{-5} %, t_R = 26.3 min). As **2** and **3** were contained in Fr. 4-1 other than Fr. 3-1, isolation of **2** and **3** from Fr. 4-1 was also performed. Fr. 4-1 (7.8 mg) was purified by reverse-phase HPLC (Develosil ODS-HG-5, φ 20 × 250 mm, 30 % aqueous MeOH, 5 mL/min, 25 °C, 215 nm, 254 nm) to afford **2** (0.7 mg, 1.1×10^{-4} %, t_R = 36.8 min) and Fr. 4-1-2. Fr. 4-1-2 (5.8 mg) was purified by reverse-phase HPLC {Develosil ODS-HG-5, φ 20 × 250 mm, 10–40 % (linear gradient for 90 min) aqueous MeOH, 5 mL/min, 25 °C, 215 nm, 254 nm} to give **3** (0.5 mg, 7.6×10^{-5} %, t_R = 26.3 min) and adenosine (**4**) (0.2 mg, 3.0×10^{-5} %, t_R = 37.9 min).

Inaoside A (1): white amorphous powder; $[\alpha]_D^{20}$ +55.7 (*c* 0.187, MeOH); IR (ZnSe-ATR) 3327 (br), 2924, 1617, 1521, 1446, 1420, 1375, 1314, 1198, 1124, 1092, 1039, 955, 905, 839, 783 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRMS (DART) *m/z* 341.1591 $[M+H]^+$ (calcd for $C_{17}H_{25}O_7^+$, Δ −0.4 mmu).

MTA (2): white amorphous powder; $[\alpha]_D^{20}$ +20.4 (*c* 0.050, MeOH); IR (ZnSe-ATR) 3132 (br), 2920, 2851, 1741, 1652, 1602, 1468, 1426, 1336, 1296, 1250, 1083, 1034, 1004, 925, 889, 850, 796, 723 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.35 (s, 1H, H8), 8.15 (s, 1H, H2), 7.26 (br s, 2H, NH_2), 5.88 (d, *J* = 5.8 Hz, 1H, H1′), 4.74 (dd, *J* = 5.4, 5.4 Hz, 1H, H2′), 4.14 (dd, *J* = 4.8, 4.0 Hz, 1H, H3′), 4.03 (m, 1H, H4′), 2.87 (dd, *J* = 13.9, 5.9 Hz, 1H, H5′a), 2.78 (dd, *J* = 13.9, 7.0 Hz, 1H, H5′b), 2.05 (s, 3H, Me-10); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.1 (C6), 152.8 (C2), 149.5 (C4), 139.9 (C8), 119.1 (C5), 87.5 (C1′), 83.8 (C4′), 72.63 (C3′), 72.61(C2′), 36.1 (C5′), 15.6 (C10). ^{13}C NMR data was based on multiplicity-edited HSQC and HMBC spectra. ^1H and ^{13}C NMR data are consistent with the reported data [18,19].; HRMS (DART) *m/z* 298.0966 $[M+H]^+$ (calcd for $C_{11}H_{16}N_5O_3S^+$, Δ −0.2 mmu).

Nicotinamide (3): white amorphous powder; IR (ZnSe-ATR) 3187 (br), 2920, 2850, 2366, 1671, 1638, 1618, 1594, 1571, 1421, 1390, 1201, 1133, 1028, 786, 773, 760, 744, 726 cm–1; ^1H NMR (500 MHz, DMSO-d_6) δ 9.02 (d, *J* = 1.5 Hz, 1H, H2), 8.70 (dd, *J* = 4.7, 1.3 Hz, 1H, H6), 8.20 (ddd, *J* = 7.9, 1.85, 1.85 Hz, 1H, H4), 8.16 (br s, 1H, NH_2), 7.59 (br s, 1H, NH_2), 7.49 (dd, *J* = 7.9, 4.8 Hz, 1H, H5). ^1H NMR data is consistent with the reported data [20].; HRMS (DART) *m/z* 123.0568 $[M+H]^+$ (calcd for $C_6H_7N_2O^+$, Δ +1.5 mmu).

Adenosine (4): white amorphous powder; $[\alpha]_D^{20}$ −55.7 (*c* 0.005, MeOH); IR (ZnSe-ATR) 3330 (br), 3197 (br), 2926, 2856, 1649, 1603, 1578, 1479, 1422, 1375, 1334, 1303, 1252, 1208, 1123, 1087, 897, 840, 797, 724, 705 cm–1; ^1H NMR (500 MHz, DMSO-d_6) δ 8.34 (s, 1H, H8), 8.13 (s, 1H, H2), 7.33 (br s, 2H, NH_2), 5.87 (d, *J* = 6.2 Hz, 1H, H1′), 5.57–5.10 (br m, 3H, OH), 4.60 (dd, *J* = 5.4, 5.4 Hz, 1H, H2′), 4.14 (dd, *J* = 4.7, 3.0 Hz, 1H, H3′), 3.96 (dd, *J* = 6.6, 3.4 Hz, 1H, H4′), 3.67 (dd, *J* = 12.3, 3.6 Hz, 1H, H5′a), 3.55 (dd, *J* = 12.1, 3.3 Hz, 1H, H5′b). ^1H NMR data is consistent with the reported data [21].; HRMS (DART) *m/z* 268.1041 $[M+H]^+$ (calcd for $C_{10}H_{14}N_5O_4^+$, Δ +0.1 mmu).

2.4. Determination of the absolute stereochemistry

2.4.1. Acid hydrolysis of *1*

Approximately 0.5 mg (1.5 μmol) of **1** was dissolved in 50 μL of 1 N aqueous HCl. The reaction mixture was stirred at 80 °C for 2 h, washed with EtOAc to separate a sugar moiety contained in the aqueous layer and the aglycon contained in the EtOAc layer. The aqueous layer was evaporated and the residue was used for the next step without further purification.

2.4.2. Preparation of thiourea *S1*

Acid hydrolysis of **1** gave a residue containing a sugar moiety. A solution of the sugar moiety of **1** and L-cysteine methyl ester hydrochloride (0.33 mg, 1.9 μmol) in pyridine (45 μL) was stirred at 60 °C for 1 h. Then a solution of *o*-tolyl isothiocyanate (0.36 mg, 2.6 μmol) in pyridine (45 μL) was added, and the resulting mixture was stirred at 60 °C for 1 h. The reaction mixture was evaporated to afford the crude material containing thiourea **S1** (Fig. S9).

2.4.3. HPLC analysis of thioureas from ribose

The crude material containing **S1** converted from **1** was analyzed by reverse-phase LC-ESI-MS (Develosil ODS-HG-5, φ 4.6 × 250 mm, 25 % aqueous acetonitrile, 0.8 mL/min, 35 °C, 254 nm, **S1**: t_R = 23.21 min). Both authentic D- and L-ribose were reacted in the same condition as described above. The absolute configuration of the sugar moiety in **1** was assigned by comparing the retention time of **S1** to those of authentic D- and L-ribose derivatives in reverse-phase LC-ESI-MS analysis (D-ribose derivative: t_R = 23.22 min; L-ribose derivative: t_R = 16.34 min) [22].

2.5. DPPH radical scavenging assay

DPPH radical scavenging assay was performed following the protocol shown below [23].

0.2 mM of the DPPH ethanol solution was prepared. The DPPH solution was placed in the dark for 2 h before use. 30 μL of the sample solutions dissolved in ethanol (final concentration: 1, 10, 50, or 100 μg/mL for **1**, 0.1, 1, 10, or 100 μg/mL for the other samples) and 120 μL of 0.1 M Tris-HCL buffer (pH 7.4) were added to a 96-well plate. Then 150 μL of DPPH solution was added to each well and mixed well by pipetting. The plate was kept in the dark for 30 min at 25 °C. Trolox was used as a positive control, ethanol was used as a negative control, and a mixture of 180 μL of ethanol and 120 μL of 0.1 M Tris-HCL buffer was used as the blank. The absorbance at 517 nm was measured using a microplate reader (Multiskan Sky, Thermo Fisher Scientific). All assays were performed at least twice to confirm the repeatability. The inhibition ratio (%) was calculated using Eq. (1):

$$Inhibition\ ratio\ (\%) = \left[\left(A_{ethanol} - A_{sample}\right)/A_{ethanol}\right] \times 100 \quad (1)$$

where $A_{ethanol}$ is the absorbance of ethanol, and A_{sample} is the absorbance of sample. The IC_{50} of each sample was calculated by plotting the DPPH radical scavenging ratio against the sample concentration. The trolox equivalent antioxidant capacity (TEAC) was obtained using Eq. (2):

$$TEAC = IC_{50}\ of\ \text{Trolox}\ [\mu M] / IC_{50}\ of\ sample\ [\mu M] \quad (2)$$

2.6. Superoxide dismutase assay

The superoxide dismutase (SOD) activity was measured using a kit (SOD Assay kit-WST, Dojindo Laboratories, Kumamoto, Japan) following the manufacturer's protocol [24].

20 μL of the sample solutions dissolved in distilled water (final concentration, 2, 10, 50, or 250 μg/mL) and 200 μL of the WST-1 solution were added to a 96-well plate. Then 20 μL of enzyme solution was added to each well and mixed well by pipetting. The plate was kept in the dark for 20 min at 37 °C. Distilled water was used as a negative control. Wells that contained distilled water instead of the enzyme solution were used as the blank sample and the blank negative control. The absorbance of the WST-1 formazan solution at 450 nm was measured using a microplate reader (Multiskan Sky, Thermo Fisher Scientific). The inhibition ratio (%) was calculated using Eq. (3):

$$Inhibition\ ratio\ (\%) = [\{(A_{NC} - \mathrm{A_{BNC}}) - (A_S - A_{BS})\}/(A_{NC} - A_{BNC})] \times 100 \quad (3)$$

where A_{NC}, A_{BNC}, A_S, and A_{BS} are the absorbance of the negative control, the blank negative control, the sample, and the blank sample, respectively. The IC_{50} of each sample was calculated from a plot of the SOD inhibition ratio against the sample concentration.

3. Results and discussion

3.1. Isolation of 1–4

Fresh fruiting bodies of *L. cremeiporus* (1.3 kg) were extracted with MeOH and the extract was concentrated and partitioned

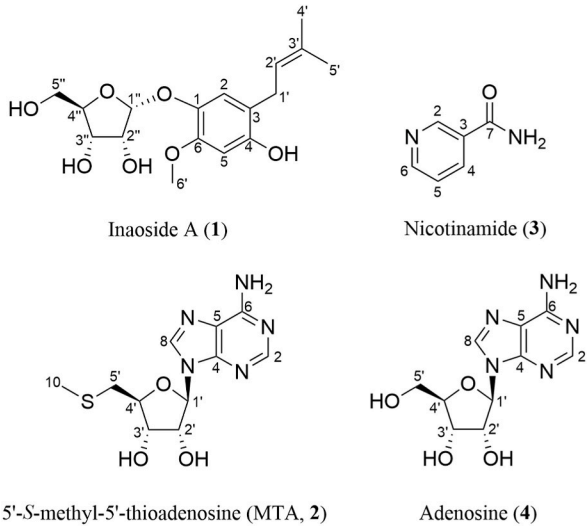


Fig. 1. Structures of inaoside A (**1**), MTA (**2**), nicotinamide (**3**), and adenosine (**4**) isolated from *L. cremeiporus*.

between water and EtOAc. The EtOAc layer was successively partitioned with *n*-hexane, CH_2Cl_2, and 60 % aqueous MeOH. The *n*-hexane and 60 % aqueous MeOH layers were not active in the DPPH radical scavenging assay at 100 μg/mL. By contrast, the CH_2Cl_2 layer exhibited DPPH radical scavenging activity at 100 μg/mL. Thus, we investigated the bioactive compounds in the CH_2Cl_2 layer. Guided by the DPPH radical scavenging assay, the CH_2Cl_2 layer was separated by SiO_2 and ODS column chromatography to afford four fractions (Fr. 3-1, Fr. 3-2, Fr. 4-1, and Fr. 4-2), which exhibiting DPPH radical scavenging activity at 100 μg/mL (Fig. S1). The new antioxidant phenolic compound inaoside A (**1**) {2.4 mg, 3.6×10^{-4} %, $[\alpha]_D^{24}$ +55.7 (*c* 0.187, MeOH)} was isolated along with three well-known bioactive compounds 5′-*S*-methyl-5′-thioadenosine (MTA, **2**: 1.2 mg, 1.8×10^{-4} %), nicotinamide (**3**: 0.7 mg, 1.1×10^{-4} %), and adenosine (**4**: 0.2 mg, 3.0×10^{-5} %) by reverse-phase HPLC purification from the above bioactive fractions (Fig. 1).

3.2. Structure analysis of *1*

The molecular formula of **1** was found to be $C_{17}H_{24}O_7$ by HRMS (DART) ($[M+H]^+$, *m/z* 341.1591, Δ −0.4 mmu). Determination of the planar structure of **1** was carried out by one- and two-dimensional NMR analysis (Fig. 2). The ^1H, ^{13}C NMR, and multiplicity-edited HSQC spectra in DMSO-d_6 showed that **1** had the following signals: two doublet methyl groups (δ_H 1.65, 1.67), one methoxy group (δ_H 3.68), four sp^3 carbons attached to oxygens (δ_C 61.6, 69.5, 71.7, 86.1), eight unsaturated carbon atoms (δ_C 101.0, 118.9, 121.3, 123.2, 130.8, 137.7, 149.1, 150.4), and one acetal carbon atom (δ_C 102.5). The IR spectrum of **1** exhibited absorption bands for hydroxy groups (3327 cm^{-1}).

An analysis of the COSY spectrum of **1** allowed us to construct two partial structures: C2–C3–C1′–C5′ and C1″–C5” (Fig. 2, Table 1). The presence of a benzene ring provided by HMBC correlations between H2/C1, C3, C4, and C6 and H5/C1, C3, C4, and C6. The presence of a tetrahydrofuran ring in **1** was established based on HMBC correlation between H1”/C4”. HMBC correlation between H1”/C1 indicated that the aromatic carbon C1 was linked to the acetal carbon C1″ via an ether bond. Moreover, C6′ methoxy group at the aromatic carbon C6 was established by HMBC correlation between Me-6’/C6. On the basis of the molecular formula and degree of unsaturation, **1** was exhibited to contain four hydroxy groups at C4, C2″, C3″, and C5″ positions. To determine the positions of substituents on the benzene ring moiety, NOE and ROE experiments in DMSO-d_6 were carried out [25–27]. NOE correlations for H2/H1′ and H1″ suggested that C1 and C3 were each connected to the aromatic carbon C2. NOE correlation for H5/Me-6′ and ROE correlation for H5/4-OH indicated that C4 and C6 were each connected to the aromatic carbon C5. Further C1–C6 connectivity was established based on the key NOE correlation for H1”/Me-6’. Additionally, C3–C4 connectivity provided by HMBC correlation between H1’/C3, and C4. From these results, the planar structure of **1** was determined to be shown in Fig. 2.

Next, the relative stereochemistry of **1** was established (Fig. 3). NOEs were observed for H1”/H2″, H1”/H3″, H2”/H5″, and H3”/H5”. These data suggested that H1″, H2″, H3″, and C5″ were oriented in one face (β-face) with respect to the tetrahydrofuran ring in **1**. Thus, **1** was determined to be α-ribofuranoside. Based on the comparison of the experimental coupling constant of **1** ($J_{1'',2''} = 4.3$ Hz) with those of methyl-α-ribofuranoside ($J_{1'',2''} = 4.3$ Hz) and methyl-β-ribofuranoside ($J_{1'',2''} = 1.2$ Hz) from the literature [28,29], the ribose moiety of **1** was confirmed to be α-ribofuranoside.

To determine the absolute stereochemistry of **1**, comparing the retention time of thiourea **S1** converted from **1** to those of authentic D- and L-ribose derivatives in reverse-phase LC-ESI-MS analysis was carried out (Fig. S9). The retention times of authentic D- and L-ribose derivatives were 23.22 and 16.34 min, respectively, and that of **S1** was 23.21 min. Therefore, ribose in **1** was determined to be D-ribose. For the above results, the absolute stereochemistry of **1** was completely established.

3.3. Antioxidant activities of *1*–*4*

The *n*-hexane, 60 % aqueous MeOH, and CH_2Cl_2 layers were subjected to the DPPH radical scavenging assay. The *n*-hexane and 60 % aqueous layers did not show DPPH radical scavenging activity, but the CH_2Cl_2 layer exhibited 9 % DPPH radical inhibition at 100 μg/mL (Fig. S1). Therefore, the CH_2Cl_2 layer was separated by SiO_2 and ODS column chromatography to afford four fractions showing DPPH radical scavenging activity at 100 μg/mL (Fr. 3-1: 21 %, Fr. 3-2: 78 %, Fr. 4-1: 19 %, and Fr. 4-2: 62 % inhibition). The

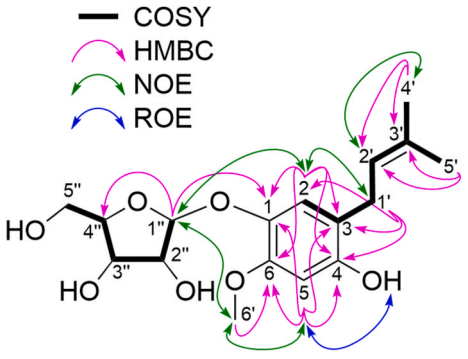


Fig. 2. Planar structure of inaoside A (**1**) constructed by two-dimensional NMR analysis (bold line, COSY; pink arrows, selected HMBC correlations; green arrows, selected NOE correlations; blue arrows, selected ROE correlations).

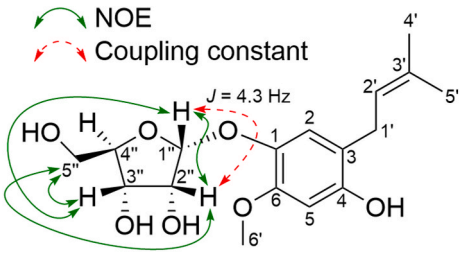
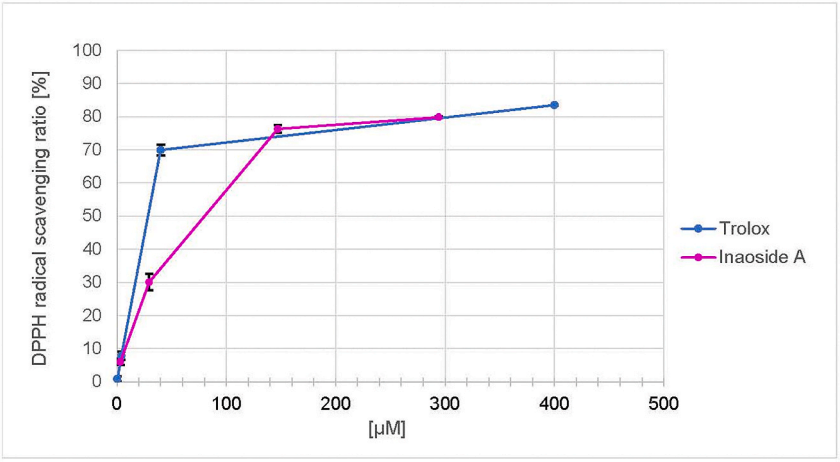


Fig. 3. The relative stereochemistry of inaoside A (**1**) constructed by one- and two-dimensional NMR analysis (green arrows, selected NOE correlations; dashed red arrow, coupling constant).



compounds	DPPH radical scavenging assay		
	radical scavenging ratio at 100 μg/mL (%)	IC_{50} (μM)	TEAC
Inaoside A (**1**)	80	79.85 ± 2.85	0.36 ± 0.013
MTA (**2**)	not tested	not tested	not tested
Nicotinamide (**3**)	4	> 819.67	
Adenosine (**4**)	not tested	not tested	not tested
Trolox	84	28.41 ± 0.42	1

Fig. 4. DPPH radical scavenging activity of inaoside A (**1**). The values are the means or the means ± standard deviations (SD) from four parallel measurements (n = 4). Values were considered significantly different at $p < 0.05$.

antioxidant activities of **1**–**4** isolated from the above bioactive fractions were investigated by the DPPH radical scavenging assay (Fig. 4) and SOD assay (Fig. S13). Compound **1** exhibited DPPH radical scavenging activity as a monophenolic compound (80 % inhibition at 100 μg/mL, IC_{50} 79.9 μM). The TEAC value of **1** was 0.36. Compound **3** did not show DPPH radical scavenging activity at 100 μg/mL. Because **2** and **4** did not dissolve in ethanol, the DPPH radical scavenging activities of these compounds could not be evaluated in our assay.

Compound **4** did not exhibit SOD activity at 250 μg/mL, whereas **1**, **2**, and **3** showed weak SOD activities (**1**: 29 %, **2**: 53 %, and **3**: 18 % inhibition at 250 μg/mL) (Fig. S13).

4. Conclusion

The structures and bioactivities of the new antioxidant phenolic compound inaoside A (**1**) and three well-known bioactive compounds 5′-*S*-methyl-5′-thioadenosine (MTA, **2**), nicotinamide (**3**), and adenosine (**4**) were established. Compounds **1**, **2**, and **4** were isolated from *L. cremeiporus* for the first time. This is the first report of the isolation of an antioxidant compound from *L. cremeiporus*. Compound **1** exhibited DPPH radical scavenging activity with an IC_{50} value of 79.9 μM. Further biological studies of **1**, including of its antioxidant, antimicrobial, anti-inflammatory, and dopamine D2 receptor agonistic activities, are in progress.

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CRediT authorship contribution statement

Atsushi Kawamura: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Aira Mizuno:** Formal analysis. **Mayuri Kurakake:** Formal analysis. **Akiyoshi Yamada:** Validation, Resources, Investigation, Formal analysis. **Hidefumi Makabe:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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