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A NOVEL DIMERIC LACTONE BIS-OSMUNDALACTONE FROM THE JAPANESE INEDIBLE MUSHROOM *PAXILLUS ATROMENTOSUS* VAR. *BAMBUSINUS*[§]

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Abstract-As a part of our systematic investigation of biologically active substances of inedible mushrooms, we studied the chemical constituents of a Basidiomycetes fungus, *Paxillus atrotomentosus* var. *bambusinus* belonging to the Paxillaceae family, and isolated a novel dimeric lactone named bis-osumundalactone (**1**). The absolute structure of compound (**1**) was determined by a combination of FAB-MS and high resolution NMR spectra with chemical reactions.

1. INTRODUCTION

We have been interested in the biologically active substances present in inedible mushroom without toxicity. Recently, we found that an inedible mushroom, *Cryptoporus volvatus* (Polyporaceae) contained a large amount of novel bitter drimane sesquiterpenoids, cryptoporic acids (CPAs) A∼G which showed strong inhibition of superoxide anion radical release.[1] *Paxillus atromentosus* belonging to the Paxillaceae family is a lignicolous inedible mushroom with a large cap and frequently appears on decayed pine trees. Previously, Steglich *et al*.[2-4] reported the isolation and structural characterization of novel leucomentin,flavomentin and

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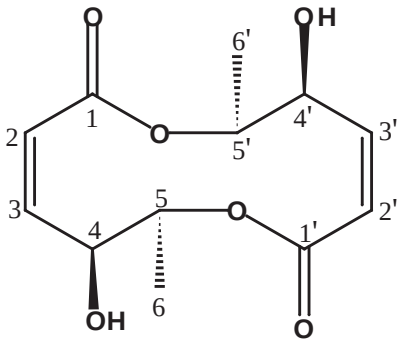
[§]Dedicated to the cerebration of 70th birthday of Professor James P. Kutney

spiromentin derivatives from the European *P. atrotomentosus.*

Recently, we have also isolated a new δ-lactone, (+)-osumundalactone (**2**) and six new spiromentins E-J from the ethyl acetate extract of the Japanese *P. atrotomentosus.*[5] An inedible mushroom *Paxillus atromentosus* var. *bambusinus* is a variety of *P. atrotomentosus,* and rarely appears on decayed bamboo. In this paper, we report the isolation and characterization of a new dimeric lactone, bis-osumundalactone (**1**) by FAB-MS, high resolution NMR spectra and chemical reactions.

Isolation of Bis-osumundalactone

The EtOAc extract of fresh material of *Paxillus atromentosus* var. *bambusinu* was subjected repeatedly to column chromatography on silica gel using $CHCl_3$-MeOH gradient and HPLC (Diol; 20%EtOAc-$CHCl_3$) to give bis-osmundalactone (**1**) with a known phenolic compound, leucomentin-3 (**3**).[3]



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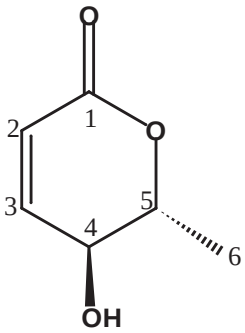

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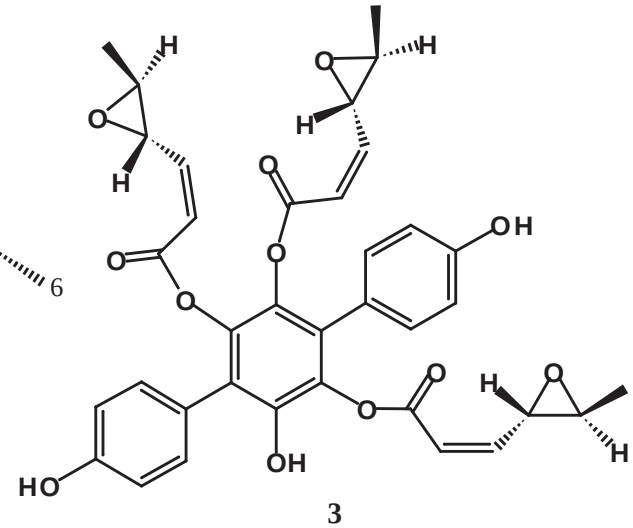

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Structural elucidation of Bis-osumundalactone

The FT-IR and UV spectral data of bis-osmundalactone (**1**) showed a hydroxy group [3408 cm^{-1}] and an α,β-unsaturated ester [1720 cm^{-1}; λ_{max} 204 nm]. The ^1H and ^{13}C NMR spectra of **1** in $CDCl_3$ indicated the presence of a secondary methyl [δ_H 1.49 (*d*, J=6.2Hz), δ_C18.1 (*q*)], an α,β-unsaturated ester [δ_H5.98 (1H, *dd*, J= 10.0, 1.8 Hz), 6.86 (1H, *dd*, J= 10.0, 2.4 Hz); δ_C120.5 (*d*), 149.0 (*d*)] and a secondary hydroxyl group [δ_H 4.25 (1H, *m*); δ_C 67.6 (*d*)]. The COSY spectrum of **1** suggested the presence of the partial structure as shown in Figure 1. The ^1H NMR, ^{13}C NMR

and COSY spectra of **1** were quite similar to those of (+)-osumundalactone (**2**) as shown in Tables 1 and 2.

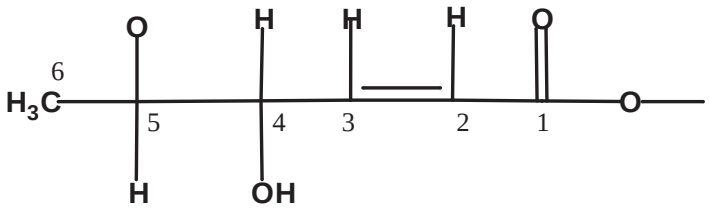

Figure 1. Partial structure of bis-osmundalactone (**1**).

Table 1. ^{1}H NMR data of bis-osmundalactone (**1**), osmundalactone (**2**) and the diacetate (**4**) of **1**[a]

H	bisosmundalactone (**1**)	osmundalactone (**2**)	diacetate (**4**)
2	5.98 (*dd*, 10.0, 1.8)	5.88 (*dd*, 10.0, 2.0)	6.11 (*dd*, 9.9, 1.6)
3	6.86 (*dd*, 10.0, 2.4)	6.85 (*dd*, 10.0, 2.3)	6.77 (*dd*, 9.9, 3.2)
4	4.25 (*m*)	4.19 (*m*)	5.28 (*ddd*, 6.6, 3.2, 1.6)
5	4.39 (*dq*, 8.8, 6.2)	4.33 (*dq*, 8.8, 6.6)	4.60 (*quint*, 6.6)
6	1.49 (*d*, 6.2)	1.42 (*d*, 6.6)	1.44 (*d*, 6.6)
2'	5.98 (*dd*, 10.0, 1.8)		6.11 (*dd*, 9.9, 1.6)
3'	6.86 (*dd*, 10.0, 2.4)		6.77 (*dd*, 9.9, 3.2)
4'	4.25 (*m*)		5.28 (*ddd*, 6.6, 3.2, 1.6)
5'	4.39 (*dq*, 8.8, 6.2)		4.60 (*quint*, 6.6)
6'	1.49 (*d*, 6.2)		1.44 (*d*, 6.6)
OH	2.83 (*br. d, J*=6.4)	4.23 (*br. d, J*=6.2)	
OAc			2.14 (*s*)

[a]Chemical shifts from TMS (multiplicity, *J* in Hz) in $CDCl_3$ and assignments from COSY spectrum

Table 2. ^{13}C NMR data of bis-osmundalactone (**1**), osmundalactone (**2**) and the diacetate (**4**) of **1**[a]

C	bisosmundalactone (**1**)	osmundalactone (**2**)	diacetate (**4**)
1	163.4 (*s*)	164.1 (*s*)	162.1 (*s*)
2	120.5 (*d*)	119.6 (*d*)	122.8 (*d*)
3	149.0 (*d*)	150.2 (*d*)	142.8 (*d*)
4	67.6 (*d*)	67.0 (*d*)	67.7 (*d*)
5	79.1 (*d*)	79.2 (*d*)	76.4 (*d*)
6	18.1 (*q*)	17.9 (*q*)	18.3 (*q*)
1'	163.4 (*s*)		162.1 (*s*)
2'	120.5 (*d*)		122.8 (*d*)
3'	149.0 (*d*)		142.8 (*d*)
4'	67.6 (*d*)		67.7 (*d*)
5'	79.1 (*d*)		76.4 (*d*)
6'	18.1 (*q*)		18.3 (*q*)
OAc			20.8 (*q*)
			170.0 (*s*)

[a]Chemical shifts from TMS in $CDCl_3$ and assignments from HMQC and HMBC spectra

The fast atom bombardment (FAB)-mass spectrometry (MS) of bis-osmundalactone (**1**) gave peaks at *m/z* 257 $[M+H]^+$ and 289 $[M+Na]^+$. The high resolution FAB-MS showed a peak at m/z 257.1034 corresponding to a molecular formula of $C_{12}H_{16}O_6$ which was two times that of osumundalactone (**2**). In the ^{13}C NMR spectrum of **1**, only 6 carbon signal were observed, suggesting that **1** might be a symmetrical dimer of **2**. Acetylation of **1** with Ac_2O and pyridine afforded diacetate (**4**) [CI-MS (CH_4) and FAB-MS: *m/z* 341 $[M+H]^+$; δ_H 2.14 (3H, *s*); δ_C 20.8 (*q*), 170.0 (*s*)] indicating the presence of two secondary hydroxyl groups in **1**. The relative structure

of **1** was inferred by the careful analysis of HMQC (^{1}H detected multiple quantum coherence), HMBC and NOESY spectra of **4**. In HMBC spectrum (Figure 2) of **4**, H-5 was correlated with C-3, C-4 and C-1'. Bis-osmundalactone (**1**) might be a C_2-symmetrical dimer, since **1** showed an optical activity {$[\alpha]_D$+27.0° ($CHCl_3$)}.

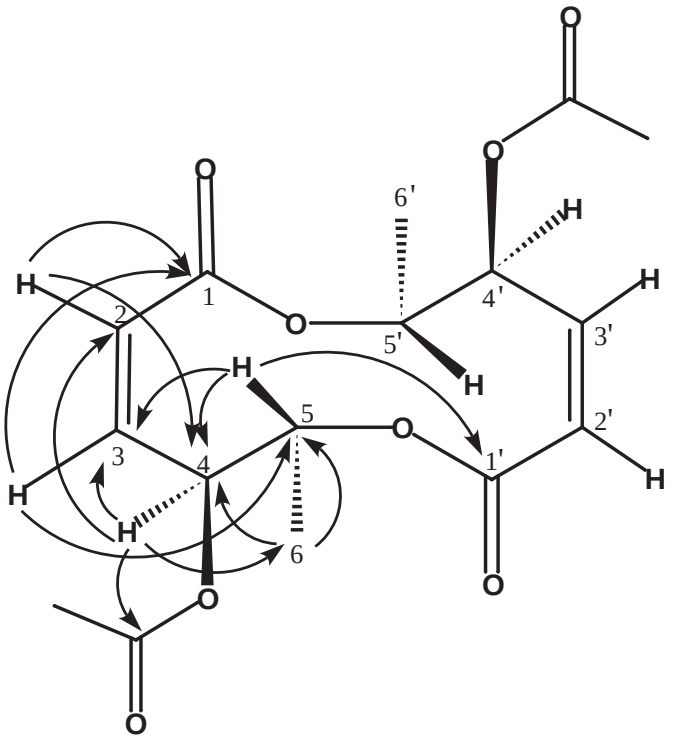

Figure 2. HMBC correlation of the diacetate (**4**)

The absolute configuration of **1** was determined by chemical correlation with (+)-osumundalactone (**2**). Hydrolysis of compound (**1**) with 10 % NaOH, followed by neutralization as usual manner gave **2**, the spectral data (^{1}H and ^{13}C NMR, IR, and MS) of which were identical with those of (+)-osumundalactone (**2**) isolated from *P. atrotomentosus*.[5] The melting point and specific rotation of the synthetic product were mp 72-74° and $[\alpha]_D^{20}$ -29.8° (*c* 0.92, $CHCl_3$), while the literature values[5] were mp 73-74° and $[\alpha]_D^{20}$ -28.8° (*c* 2.35, $CHCl_3$). In conclusion, the absolute configuration of bis-osmundalactone (**1**) was established as (4*S*, 5*R*, 4'*S*, 5'*R*)-configurations.

Table 3 showed the distribution of osumundalactone (**2**) and its related compounds in three *Paxillus* species. Both Japanese and European *P. atrotomentosus* produce leucomentin-3 (**3**).[3] It is noteworthy that the Japanese *Paxillus atromentosus* var. *bambusinus* elaborates the characteristic bis-osmundalactone (**1**) without the monomeric osmundalactone (**2**).

Table 3. Distribution of bis-osmundalactone (**1**), osmundalactone (**2**) and leucomentin-3 (**3**) in *Paxillus* species

species	(**1**)	(**2**)	(**3**)
Japanese *P. atrotomentosus*[5]		++++*	+++
European *P. atrotomentosus*[2-4]			+++
P. atromentosus var. *bambusinus*	++		+++

*The symbol (++ ~ ++++) was shown on the base of a concentration of products

EXPERIMENTAL

IR spectra were measured on a Jasco FT-IR 500 spectrophotometer. ^{1}H and ^{13}C NMR were recorded on a Varian untiy 600 (^{1}H; 600 MHz, ^{13}C; 150 MHz) or a Varian Unity 200 (^{1}H; 200 MHz, ^{13}C; 50 MHz) spectrometer. The solvent used for NMR spectra was $CDCl_3$ unless otherwise stated. MS spectra were measured on a JEOL JMS HX-100 or a JEOL AX-500 spectrometer. The specific rotation was taken on a JASCO DIP-140 polarimeter. Silica gel 60 for column chromatography was purchased from Merk.

Isolation of Bis-osmundalactone (1) The fresh materials (1.51 kg) of *Paxillus atromentosus* var. *bambusinus* collected in Nishinomiya-city, Hyougo prefecture, Japan, in July 1997, was extracted with EtOAc (3 L) for two times for 1 day at rt. After filtration, the brown solution was evaporated *in vacuo* to afford dark brown residue (55.74 g). A part of residue (4.0 g) was chromatographed on silica gel (100g) with a gradient solvent system of $CHCl_3$-EtOAc increasing the amount of 5% portion EtOAc stepwise to give 153 fractions. 50% EtOAc-$CHCl_3$ eluate (Fr. 64-83) was evaporated in *vacuo* to afford the crude oil (218.3 mg), which was further purified by reversed HPLC (Nucleosil $5C_{18}$; 50%H_2O-MeCN) to give leucomentin-3 (**3**)[3] (174 mg), the spectral data (IR, ^{1}H and ^{13}C NMR) of which were identical with those of leucomentin-3.[3] A

remaining crude oil (51.74 g) was chromatographed on silica gel (600 g) with a gradient solvent system of $CHCl_3$-MeOH increasing the amount of 1% portion MeOH stepwise to give 35 fractions. 5% MeOH-$CHCl_3$ eluate (Fr. 14-16) was evaporated in *vacuo* to afford the crude oil (4.02 g), a part (200 mg) of which was chromatographed on Diol with $CHCl_3$-EtOAc gradient, followed by HPLC (Diol; 20%EtOAc-$CHCl_3$) to give bis-osmundalactone (**1**) (23 mg).

Bis-osmundalactone (1) colorless oil, $[\alpha]_D^{20}$ +27.0° (*c* 1.00, $CHCl_3$); CI-MS (CH_4): *m/z* 257 $[M+H]^+$; FAB(+)-MS: *m/z* 257 $[M+H]^+$, 289 $[M+Na]^+$; HRFAB(+)-MS: m/z 257.1034 $[M+H]^+$, $C_{12}H_{17}O_6$ requires 257.1025; FT-IR (KBr)cm^{-1}: 3408 (OH), 1720 (COO); UV (EtOH) λ_{max}nm (log ε); 204.0 (4.33); ^1H and ^{13}C NMR($CDCl_3$)(Tables 1 and 2).

Acetylation of Compound (1) A solution of compound (**1**) (10 mg) in pyridine (1 mL) was treated with acetic anhydride (1 mL). The mixture was stirred at rt overnight. Water was added and the mixture was extracted with $CHCl_3$. The organic phase was washed with 1N HCl, 5% $NaHCO_3$ solution and brine, dried ($MgSO_4$), and evaporated to give a residue which was purified by a silica gel column chromatography with hexane-AcOEt gradient to afford a diacetate (**4**) (7 mg) as a colorless oil; $[\alpha]_D^{20}$ +38.9° (*c* 0.75, $CHCl_3$); CI-MS (CH_4): *m/z* 341 $[M+H]^+$, 193, 171, 126, 111 (100%), 84; FAB-MS: *m/z* 341 $[M+H]^+$, 363 $[M+Na]^+$; FT-IR (KBr)cm^{-1}: 1734 (COO), 1636 (C=C); UV (EtOH) λ_{max}nm (log ε); 205.5 (4.33); ^1H and ^{13}C NMR($CDCl_3$)(Tables 1 and 2).

Chemical conversion of bis-osmadulactone (1) into osmadulactone (2) To a solution of compound (**1**)(10 mg) in MeOH (3 mL) was added 10% NaOH solution (0.5 mL). The mixture was stirred at rt for 12 h. The reaction mixture was poured in 10% HCl solution (20 mL) and stirred at rt for 1 h. The mixture was extracted with $CHCl_3$ (50 mL x 3). The organic layer was washed with brine, dried ($MgSO_4$) and evaporated under reduced pressure to give crude product (14 mg) which was recrystallized from EtOAc-Et_2O to furnish osmadulactone (**2**) (7 mg) as colorless needles; mp 81.0-83.0° (lit.,[5] mp 81.5-82.5°], $[\alpha]_D^{20}$ +71.5° (*c* 0.70, $CHCl_3$) {lit.,[5] $[\alpha]_D$ +70.7° (*c*1.27, $CHCl_3$)}; HRCI-MS; m/z 129.0558 $[M+H]^+$, $C_{12}H_{17}O_6$ requires 257.1025; CI-MS (CH_4): *m/z* 129 ($[M+H]^+$, 100%), 111, 103, 87. The spectral data (IR, UV, ^1H and ^{13}C NMR) of synthetic product were identical with those of natural osmadulactone (**2**).[5]

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