HETEROCYCLES, Vol. 87, No. 7, 2013, pp. 1481 - 1491. © 2013 The Japan Institute of Heterocyclic Chemistry
Received, 15th March, 2013, Accepted, 13th May, 2013, Published online, 17th May, 2013
DOI: 10.3987/COM-13-12707

PALMAERINS A-D, NEW CHLORINATED AND BROMINATED DIHYDROISOCOUMARINS WITH ANTIMICROBIAL AND PLANT GROWTH REGULATING ACTIVITIES FROM DISCOMYCETE *LACHNUM PALMAE*

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Abstract – A new chlorinated dihydroisocoumarin, palmaerin A (**1**), and three new brominated dihydroisocoumarins, palmaerins B (**2**), C (**3**), and D (**4**), were isolated from the supernatant of the culture broth containing KCl and KBr of discomycete *Lachnum palmae*, respectively. The structures of **1**-**4** were elucidated by spectroscopic data and X-ray diffraction analysis. Compounds **2** and **3** exhibited weak antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus,* while compounds **1**-**3** showed plant growth regulating activity against *Lepidium sativum*.

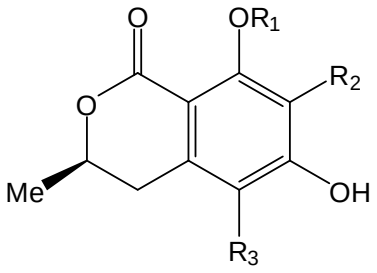
INTRODUCTION

In previous reports, several new bioactive substances such as scyphostatin[1] and F-10863s[2,3] which have enzyme inhibition activity, have been isolated from fungi of the order Leotiales, Discomycetes. Nevertheless, the members of it have been underutilized for microbial screenings.[4] Therefore, we noticed discomycetes as research source for new bioactive substances. In our screening for antimicrobial activity of various strains of discomycetes in the National Museum of Nature and Science, *Lachnum palmae* was selected. The genus of *Lachnum* is known to embrace about 250 species and yet more members have been

added to science,[5] and the antimicrobial and nematicidal pentaketide compounds have been isolated from *Lachnum papyraceum*.[6-8]

In our previous investigation, we found two new chlorinated dibenzo-α-pyrones, palmariols A and B, and two new chlorinated cyclopentenones, palmaenones A and B which have antimicrobial activity, have been isolated from the mycelial extracts and culture broth of *L. palmae*, respectively.[9,10] On the other hand, chlorinated or brominated compounds have been isolated from some discomycetes. For example, chloromonilicin and bromomonilicin as self-growth inhibitors,[11,12] and chloromonilinic acids A and B[13] as antifungal compounds were isolated from *Monilinia fructicola*. KS-504a-e containing seven chlorine atoms were isolated from *Mollisia ventosa*.[14,15] Furthermore, chlorinated antifungal compounds, mollisins A and B were isolated from *Mollisia caesia*, and this discomycete has been shown to have chloroperoxidase which catalyze the chlorination.[16,17] These reports suggest that *L. palmae* which belongs to the same order as them has also haloperoxidases for halogenation.

So in order to research other halogenated compounds from *L. palmae*, KCl or KBr was added to the culture media. In this paper, we describe the isolation and structure elucidation of four new halogenated dihydroisocoumarins **1-4** from the supernatant of the culture broth containing KCl or KBr of *L. palmae* and their antimicrobial activity and plant growth regulating activity.



1: R_1=Me, R_2=R_3=Cl

2: R_1=H, R_2=R_3=Br

3: R_1=Me, R_2=R_3=Br

4: R_1=R_2=H, R_3=Br

Figure 1. Structures of **1-4**

RESULTS AND DISCUSSION

The KCl- or KBr-containing culture broth of *L. palmae* was centrifuged. The supernatants were extracted with EtOAc. The EtOAc-soluble portions were subjected to silica gel column chromatography and reversed-phase HPLC. As results of these, a new chlorinated dihydroisocoumarin, palmaerin A (**1**) was obtained together with known chlorinated cyclopentenes, palmaenones A and B[10] from the KCl-containing culture media, while three new brominated dihydroisocoumarins, palmaerins B-D (**2-4**) were obtained from the KBr-containing culture media.

Palmaerin A (**1**) showed pseudomolecular ion peaks at *m/z* 275 $(M-H)^-$, 277 $(M+2-H)^-$, and 279 $(M+4-H)^-$ (10:6:1) in the ESI-MS, indicating the presence of two chlorine atoms in **1**. The molecular formula of **1**

was deduced as $C_{11}H_{10}{}^{35}Cl_2O_4$ from HRESI-MS [*m/z* 274.9885 $(M-H)^-$, Δ +0.7 mmu]. ^{1}H NMR spectrum of **1** was almost the same as 5,7-dichloro-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin[18] except a methoxy group at δ_H 3.86 (3H, s). The IR spectrum indicated the presence of hydroxy group (3241 cm^{-1}) and α,β-unsaturated lactone (1698 and 1266 cm^{-1}), and UV absorptions at 226, 252, and 311 nm were observed. These data were almost similar to those of 5,7-dichloro-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin.[19] The gross structure of **1** was deduced from detailed analysis of the ^{13}C and ^{1}H NMR data (Tables 1 and 2) aided by 2D NMR experiments (^{1}H-^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC)). ^{1}H NMR spectrum at δ_H 1.42 (3H, d, J = 6.3 Hz) implied the presence of one methyl group. Two double doublet with J value of 16.9 Hz between δ_H 2.73 and δ_H 3.20 indicated the existence of nonequivalent methylene protons. The ^{13}C NMR data indicated that **1** possessed one unsaturated carbonyl carbon, six aromatic carbons, one oxymethine carbon, one methoxy carbon, one methylene carbon, and one methyl carbon. The ^{1}H-^{1}H COSY connectivities between H-3 (δ_H 4.55) and methyl proton (δ_H 1.42) at C-3 as well as the HMBC correlations of the methyl proton to C-3 (δ_C 74.4) and C-4 (δ_C 34.6) indicated that the methyl group was connected to C-3, which was connected to C-4. The HMBC correlations of H_2-4 (δ_H 2.73 and 3.20) to C-4a (δ_C 140.2), C-5 (δ_C 116.3), and C-8a (δ_C 117.5) revealed that C-4 was connected to C-4a which is one of aromatic carbons. The HMBC correlations of the methoxy proton (δ_H 3.86) to C-8 (δ_C 159.5) indicated that the methoxy group was connected to C-8 (Figure 2). Comparison of the ^{13}C NMR data of compound **1** with 3,4-dihydro-6-hydroxy-8-methoxy-3-methylisocoumarin[20] revealed the presence of two chlorine atoms at C-5 and C-7. The configuration of methyl group at C-3 was determined by comparison of the optical rotation data of the previous report,[21] and revealed that compound **1** has *R*-configuration. Thus the structure of palmaerin A was assigned as **1**.

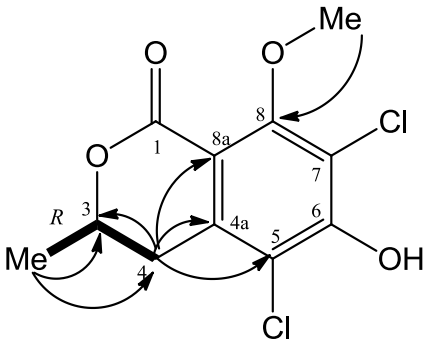


Figure 2. 2D NMR correlations of **1**

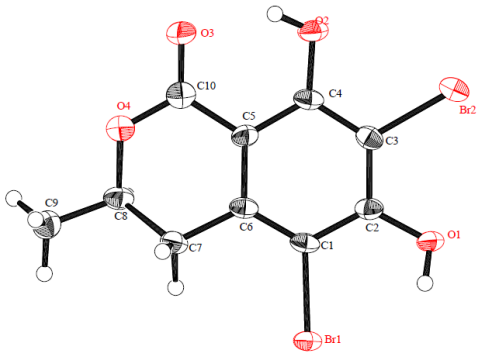


Figure 3. ORTEP drawing derived from X-ray crystal structural analysis of **2**

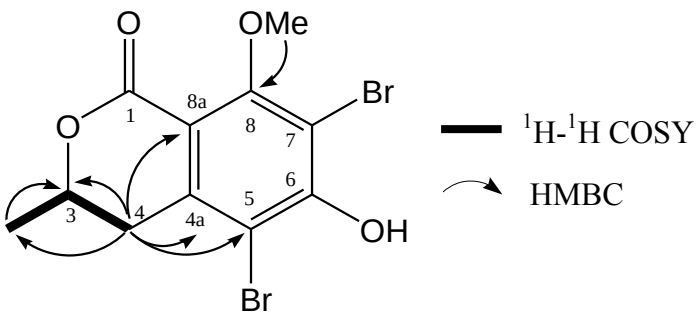


Figure 4. The HMBC correlations of **3**

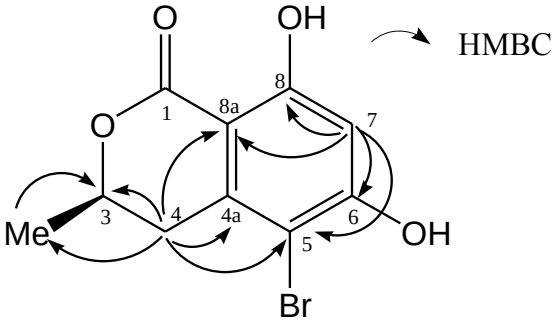


Figure 5. The HMBC correlations of **4**

Palmaerin B (**2**) showed no methoxy group in the ^{1}H NMR spectrum (Table 2), but the signal pattern was the same as **1**. In the ESI-MS, specific pseudomolecular ion peaks at *m/z* 349 $(M-H)^-$, 351 $(M+2-H)^-$, and 353 $(M+4-H)^-$ (1:2:1) were observed, indicating the presence of two bromine atoms in **2**. The molecular formula of **2** was deduced as $C_{10}H_8{}^{79}Br_2O_4$ from HRESI-MS [*m/z* 348.8717 $(M-H)^-$, Δ +0.6 mmu]. The IR and UV spectra of **2** were similar to those of **1**. The gross structure of **2** was deduced from detailed analysis of the 2D NMR experiments (^{1}H-^{1}H COSY, HMQC, and HMBC). As a result of it, it was revealed that bromine atoms were connected to C-5 and C-7. The absolute configuration of methyl group at C-3 was *R*-configuration by the single-crystal X-ray diffraction analysis of **2** (Figure 3). Thus the structure of palmaerin B was determined as **2**.

Palmaerin C (**3**) showed pseudomolecular ion peaks at *m/z* 363 $(M-H)^-$, 365 $(M+2-H)^-$, and 367 $(M+4-H)^-$ (1:2:1) in the ESI-MS, indicating the presence of two bromine atoms in **3**, and the molecular formula of **3** was deduced as $C_{11}H_{10}{}^{79}Br_2O_4$ from HRESI-MS [*m/z* 362.8877 $(M-H)^-$, Δ +0.9 mmu]. Considered that the ^{1}H NMR spectrum (Table 2) of **3** indicated the presence of methoxy group at δ_H 3.85 (3H, s), it was suggested that **3** possesses a methoxy group instead of a hydroxy group of **2**. The detailed structure of **3** was determined from analysis of ^{13}C (Table 1) and 2D NMR experiments (^{1}H-^{1}H COSY, HMQC, and HMBC). As a result of it, the HMBC correlation of the methoxy proton (δ_H 3.85) to C-8 (δ_C 161.1) was observed, indicating methoxy group was connected to C-8 (Figure 4). Furthermore, chemical shift values at C-5 (δ_C 107.9) and C-7 (δ_C 107.1) were shifted to higher field than those of **1**, indicating existence of two bromine atoms at C-5 and C-7. The configuration of methyl group at C-3 was determined by comparison of the optical rotation data of the previous report[21] as *R*-configuration. Thus the structure of palmaerin D which is the brominated derivative of **1** was determined as **3**.

Palmaerin D (**4**) showed one singlet peak at δ_H 6.47 (1H, s) in the ^{1}H NMR spectrum (Table 2). Also the presence of one bromine atom in **4** was indicated by ESI-MS (*m/z* 271 $[M-H]^-$ and 273 $[M+2-H]^-$ (1:1)), and the molecular formula was deduced as $C_{10}H_9{}^{79}BrO_4$ from HRESI-MS [*m/z* 270.9610 $(M-H)^-$, Δ +0.4 mmu]. These results suggest that **4** is the debromoderivative of **2**. The gross structure of **4** was determined

Table 1. ^{13}C NMR Data of Palmaerins A-D (**1**-**4**) in Acetone-d_6[a]

	1	**2**	**3**	**4**
1	161.6, C	170.8, C	161.7, C	170.9, C
3	74.4, CH	76.4, CH	74.3, CH	76.2, CH
4	34.6, CH	36.2, CH	37.7, CH	36.2, CH
4a	140.2, C	141.0, C	142.9, C	142.5, C
5	116.3, C	102.9, C	107.9, C	103.3, CH
6	155.4, C	159.4, C	157.4, C	162.5, C
7	113.9, C	98.4, C	107.1, C	103.3, C
8	159.5, C	161.5, C	161.1, C	164.7, C
8a	117.5, C	103.4, C	114.1, C	102.4, C
Me-3	21.2, CH_3	21.2, CH_3	21.2, CH_3	21.3, CH_3
MeO-8	62.6, CH_3		62.6, CH_3	

[a]δ_C in ppm.

Table 2. ^{1}H NMR Data of Palmaerins A-D (**1**-**4**) in Acetone-d_6[a]

	1	**2**	**3**	**4**
1				
3	4.55, m	4.74, m	4.54, m	4.70, m
4	2.73, dd (11.5, 16.9) 3.20, dd (2.9, 16.9)	2.79, dd (11.7, 17.0) 3.22, dd (3.4, 17.0)	2.72, dd (11.5, 16.9) 3.20, dd (2.9, 16.9)	2.76, dd (11.6, 17.0) 3.22, dd (3.3, 17.0)
4a				
5				
6				
7				6.47, s
8				
8a				
Me-3	1.42, d (6.3)	1.48, d (6.4)	1.42, d (6.3)	1.47, d (6.3)
MeO-8	3.86, s		3.85, s	

[a]δ_H in ppm.

from detailed analysis by 2D NMR experiments (^{1}H-^{1}H COSY, HMQC, and HMBC) (Figure 5). The HMBC correlations of H-7 (δ_H 6.47) to C-5 (δ_C 103.3), C-6 (δ_C 162.5), C-8 (δ_C 164.7), and C-8a (δ_C 102.4) and comparison of the ^{13}C NMR data of **4** with (*R*)-(-)-5-chloro-3,4-dihydro-6,8-dihydroxy-3-methyl-1*H*-2-benzopyran-1-one[21] revealed that compound **4** has one bromine atom at C-5. The configuration of methyl group at C-3 was determined as *R*-configuration by comparison of optical rotations with compounds **1**-**3** and the previous report.[22] Thus the structure of palmaerin D was assigned as **4**.

Palmaerins A-D (**1**-**4**) should be pentaketides, and it is suggested that the origin of these dihydroisocoumarins and cyclopentenes such as palmaenones A and B is a common six-membered intermediate.[18] A lot of isocoumarins have been obtained from various plants and fungi as secondary metabolites, and some of them possess interesting bioactivities. For example, 5-methylmellein obtained from *Fusicoccum amygdari* Del. inhibits the germination of spores of fungi, while it showed no phytotoxic activity. 6-Methoxymellein was isolated from fungi *Sporormia bipartis* Cain and *Sporormia affinis* Sacc., and was also produced by a carrot inoculated with fungi, is thought to be a phytoalexin. Sclerin, sclerotinins A and B promote remarkably both germination and elongation of rice, caster bean, mung bean, and other plants.[23]

Table 3. Antimicrobial Activities of Palmaerins A-D (**1**-**4**)

Test organism	Inhibition zone (mm)			
	1	**2**	**3**	**4**
Bacteria				
Bacillus subtilis	11*	14	16*	-
Staphylococcus aureus	11	17	13	-
Escherichia coli	-	-	-	-
Fungi				
Penicillium sp.	14*	30*	-	23*
Botrytis sp.	-	-	-	-
Mucor rouxii	-	-	-	-
Yeast				
Candida utilis	-	-	-	-

Concentrations tested: 1 μmol/paper disc (i.d. 8 mm), -: No inhibition zone, *: Dim inhibition zone.

Hence, antimicrobial activity and plant growth regulating activity of palmaerins A-D (**1**-**4**) were evaluated. As a result, compounds **1**-**3** showed antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Table 3). Compounds **2** and **3** exhibited stronger activity than **1**, indicating that bromine atom plays an important role in antibacterial activity. On the other hand, compounds **1**-**3** exhibited plant growth regulating activity against *Lepidium sativum* (Figure 6): at high concentrations, the growth of roots of *L. sativum* was significantly inhibited, contrary to this, at low concentrations, **1**-**3** promoted the growth of those. But this auxin-like activity of palmaerin D (**4**) was weaker than those of **1**-**3**. These results suggest

that halogen atoms play an important role in showing plant growth regulating (auxin-like) activity against *L. sativum*.

Together with the fact that *L. palmae* was isolated from decaying leaves of *Livistona*, this result implied that dihydroisocoumarins effect on the host plant positively. Furthermore, it was suggested that production of potent antimicrobial palmaenones enable *L. palmae* itself and host plant to protect from other fungi's threat. So it is possible that *L. palmae* is endophytic fungus which has another role as latent saprotroph. Actually, several fungi which have dual roles as endophyte and saprotroph have been already reported.[24]

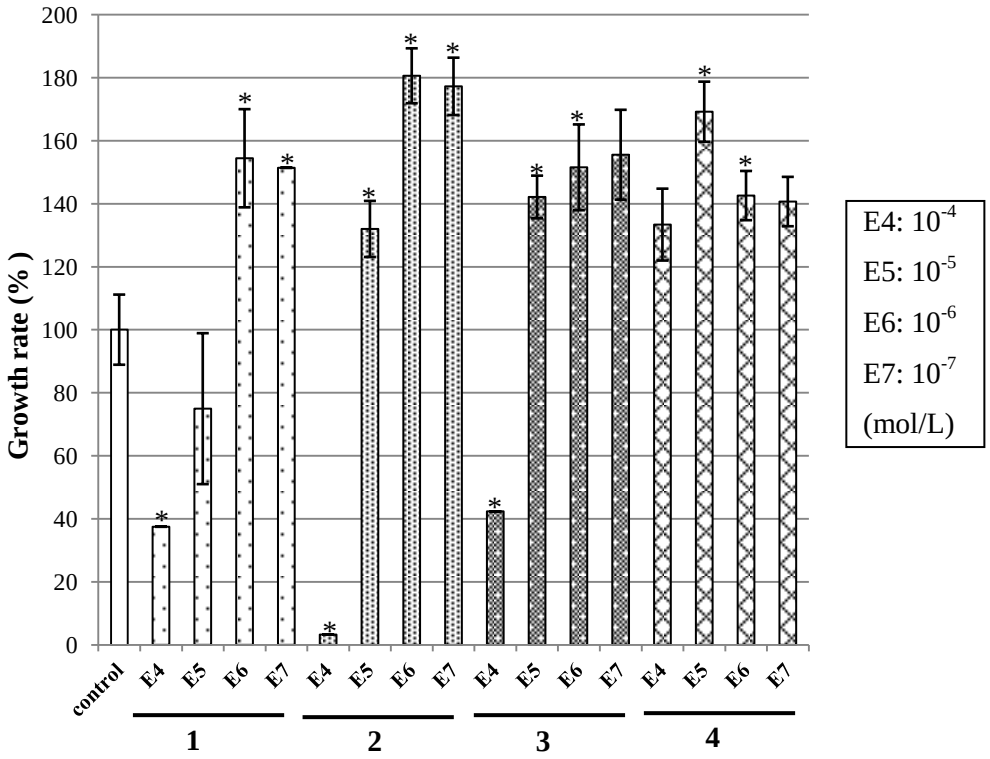


Figure 6. Growth regulating activities of palmaerins A-D (**1-4**) against *Lepidium sativum*
*: Significant difference (P<0.05)

$$\text{Growth rate} = \frac{\text{Length of the treated plants}}{\text{Length of the control plants}} \times 100$$

EXPERIMENTAL

General Procedures

Optical rotations were measured with a Jasco DIP-370 with a cell path of 1 cm. UV spectra were obtained with a HITACHI U-2000A spectrometer. IR spectra were obtained with a JASCO FT/IR-300 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker Avance-500 spectrometer in

acetone-d_6. The resonances of acetone-d_6 at δ_H 2.00 and δ_C 30.3 were used as internal references for the ^{1}H and ^{13}C NMR spectra, respectively. ESI-MS and HRESI-MS spectra were recorded with a Waters Synapt G2 mass spectrometer.

Fungal Material

The isolated NBRC-106495, deposited in the National Institute of Technology and Evaluation, Biological Resource Center (NBRC) was obtained by a single ascospore isolation using a Skerman's micromanioulator from an apothecium of *Lachnum palmae* (Kanouse). Spooner produced on the decaying leaves of *Livistona*, collected in Suzaki, Shimoda, Shizuoka Prefecture in July, 2004. The specimen was preserved as TNS-F-11197 in the National Museum of Nature and Science. The isolated TNS-F-11197 was grown and kept on potato dextrose agar (PDA, Nissui).

Fermentation

L. palmae was inoculated in 100 mL Erlenmeyer flasks (×5) containing 30 mL of seed medium (PYG: polypeptone (1%), yeast extract (0.5%), glucose (2%), and KCl or KBr (0.5%) in deionized water, pH was adjusted to 7.5 before autoclaving). After incubation on a rotary shaker at 120 rpm, 25 °C for 14 days, each seed culture was transferred into 500 mL Sakaguchi flasks (×20) containing 300 mL of PYG medium and incubated on rotary shaker at 120 rpm, 25 °C for 1 week. The culture broth was centrifuged at 7,500 rpm for 15 min.

Extraction and Isolation

The supernatant filtrate of *L. palmae* culture broth (6 L) was extracted with EtOAc and evaporated to dryness *in vacuo* at 37 °C. When KCl was added to the culture media, the EtOAc–soluble portion (462.9 mg) was subjected to silica gel column chromatography (ϕ1.1×35 cm, *n*-hexane/acetone, 99:1→0:100). A fraction (38 mg) in which characteristic peaks of palmaenone derivatives were observed, was applied to silica gel column chromatography (ϕ1.1×22 cm, *n*-hexane/acetone, 80:20→0:100) to yield palmaerin A (**1**) (18.7 mg). When KBr was added to the culture media, the EtOAc–soluble portion (253.7 mg) was subjected to silica gel column chromatography (ϕ1.1×22 cm, *n*-hexane/acetone, 99:1→0:100). A fraction (127.6 mg) in which characteristic peaks of dihydroisocoumarin derivatives were observed by the ^{1}H NMR spectra, was applied to silica gel column chromatography (ϕ1.1×22 cm, *n*-hexane/acetone, 95:5→0:100) and the fraction (63.6 mg) eluted with *n*-hexane/acetone (80:20→75:25) was further separated by reversed-phase HPLC (TSK-gel ODS-80Ts, TOSOH ϕ7.8×300 mm, flow rate 2.0 mL/min; MeOH:H_2O, 70:30 (0 min)→90:10 (30 min)→100:0 (30 min)) to yield palmaerin B (**2**) (0.9 mg),

palmaerin C (**3**) (11.9 mg), and palmaerin D (**4**) (5.0 mg).

Palmaerin A (5,7-dichloro-3,4-dihydro-6-hydroxy-8-methoxy-3-methylisocoumarin) (1): Colorless amorphous solid; $[\alpha]_D^{24}$ -114° (*c* 1.0 in acetone); UV λ_{max} (MeOH) nm (log ε) 226 (4.09), 252 (3.92), 311 (3.84); IR (KBr) ν_{max} cm^{-1} 3241, 1698, 1266; ^1H and ^{13}C NMR (Table 1); HMBC correlations (acetone-d_6, H/C): 4/3, 4/4a, 4/5, 4/8a, 4/Me-3, Me-3/3, Me-3/4, MeO-8/8; ESI-MS (negative ion) *m/z*: 275 $[M-H]^-$, 277 $[M+2-H]^-$, 279 $[M+4-H]^-$ (10:6:1); HRESI-MS (negative ion) *m/z*: 274.9885 $[M-H]^-$ (Calcd for $C_{11}H_9{}^{35}Cl_2O_4$: 274.9878).

Palmaerin B (5,7-dibromo-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin) (2): Colorless crystal; $[\alpha]_D^{24}$ -47.7° (*c* 1.0 in acetone); UV λ_{max} (MeOH) nm (log ε) 224 (4.69), 250 (4.50), 317 (4.65); IR (KBr) ν_{max} cm^{-1} 3332, 1713, 1249; ^1H and ^{13}C NMR (Table 1); HMBC correlations (acetone-d_6, H/C): 4/3, 4/4a, 4/5, 4/8a, 4/Me-3, Me-3/3, Me-3/4; ESI-MS (negative ion) *m/z*: 348 $[M-H]^-$, 350 $[M+2-H]^-$, 352 $[M+4-H]^-$ (1:2:1); HRESI-MS (negative ion) *m/z*: 348.8717 $[M-H]^-$ (Calcd for $C_{10}H_7{}^{79}Br_2O_4$: 348.8711).

Palmaerin C (5,7-dibromo-3,4-dihydro-6-hydroxy-8-methoxy-3-methylisocoumarin) (3): Colorless amorphous solid; $[\alpha]_D^{24}$ -99.4° (*c* 1.0 in acetone); UV λ_{max} (MeOH) nm (log ε) 220 (4.42), 246 (4.27), 313 (4.63); IR (KBr) ν_{max} cm^{-1} 3244, 1697, 1259; ^1H and ^{13}C NMR (Table 1); HMBC correlations (acetone-d_6, H/C): 4/3, 4/4a, 4/5, 4/8a, 4/Me-3, Me-3/3, Me-3/4, MeO-8/8; ESI-MS (negative ion) *m/z*: 363 $[M-H]^-$, 365 $[M+2-H]^-$, 367 $[M+4-H]^-$ (1:2:1); HRESI-MS (negative ion) *m/z*: 362.8877 $[M-H]^-$ (Calcd for $C_{11}H_9{}^{79}Br_2O_4$: 362.8868).

Palmaerin D (5-bromo-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin) (4): Colorless amorphous solid; $[\alpha]_D^{24}$ -61.4° (*c* 1.0 in acetone); UV λ_{max} (MeOH) nm (log ε) 218 (4.35), 267 (3.99), 314 (4.15); IR (KBr) ν_{max} cm^{-1} 3254, 1697, 1245; ^1H and ^{13}C NMR (Table 1); HMBC correlations (acetone-d_6, H/C): 4/3, 4/4a, 4/5, 4/8a, 4/Me-3, 7/5, 7/6, 7/8, 7/8a, Me-3/3, Me-3/4; ESI-MS (negative ion) *m/z*: 271 $[M-H]^-$, 273 $[M+2-H]^-$ (1:1); HRESI-MS (negative ion) *m/z*: 270.9610 $[M-H]^-$ (Calcd for $C_{10}H_8{}^{79}BrO_4$: 270.9606).

Single-crystal X-ray crystallography of palmaerin B (2) Suitable colorless platelets of **2** were obtained from a solution of acetone. The crystal (0.200×0.150×0.030 mm) belongs to the monoclinic system, space group $P2_1$ (#4), with a = 8.7403(7) Å, b = 7.0111(6) Å, c = 9.0846(7) Å, β = 97.334(2)°, V = 552.14(8) $Å^3$, Z = 2, D_{calcd} = 2.117 g/cm^3, and (Mo$K\alpha$) = 73.568 cm^{-1}. Intensity data were measured on a Rigaku RAXIS-RAPID diffractometer up to $2\theta_{max}$ of 55.0°. All 6774 reflections were collected. The structure was solved by direct methods (SHELX97) and refined with full-matrix least-squares on F^2 procedure. Non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were located by different Fourier techniques and refined with isotropic thermal parameters. The refined structural model converged to a final R1 = 0.0447; wR2 = 0.1223; Flack parameter (Friedel pairs = 1155) = 0.06(2) for

2524 observed reflections [I > 2.00σ(*I*)] and 148 variable parameters. Crystallographic data of **2** have been deposited with the Cambridge Crystallographic Data Centre (deposit No. CCDC 929142). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2, 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk).

Plant growth regulating activity assay

Filter discs (33 mm diameter) bearing test solutions (final concentration, 10^{-4}-10^{-7} mol/L) were placed in dishes, and wetted with 500 μL of water containing 0.01% Tween 20. Seven seeds of the plant (*Lepidium sativum*) were put on each disc. After incubation at 25 °C for 24 h in the dark, length of the roots was measured, and compared with a negative control. The assays were carried out in duplicate.

Antimicrobial assay

Antimicrobial activity against two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), one gram-negative bacteria (*Escherichia coli*), three fungi (*Penicillium* sp., *Botrytis* sp., and *Mucor rouxii*), and one yeast (*Candida utilis*) were tested by plate diffusion assay using 8 mm paper disc. Palmaerins A-D (**1**-**4**) solutions (1 μmol/50 μL) were prepared by dissolving each compound in acetone. Each adjusted solution (50 μL) was added to paper disc, and the paper discs were dried. The paper discs were set on the agar plate suspended tested microorganisms. After cultivating microorganisms at 37 °C for 24 h (bacteria) or at 24 °C (fungi and yeast) for 72 h, the strength of antimicrobial activity was evaluated by measuring the diameter length of inhibition zone (mm).

ACKNOWLEDGEMENT

This work was partly supported by Grants-in-Aid for the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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