Isolation, Structure Elucidation, and Chemical Derivatization of a New Cyclic Bisbibenzyl Dimer, Pusilatin E, from the Liverwort *Riccardia multifida* subsp. *decrescens*

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Received July 26, 1996[⊗]

A new cyclic bisbibenzyl dimer (**1**) has been isolated from the MeOH extract of the liverwort *Riccardia multifida* subsp. *decrescens*, together with two previously known bisbibenzyls [riccardin A (**2**) and marchantin I (**3**)] and a norneolignan [egonol 2-methylbutanoate (**4**)]. Extensive ^{1}H- and ^{13}C-NMR spectral measurements and chemical derivatization allowed the structure of dimeric riccardin A to be defined; it has been named pusilatin E (**1**).

Liverworts (Hepaticae) are known to be rich sources of both terpenoids and aromatic compounds, with interesting biological activities. The Hepaticae occasionally produce their own peculiar phenolic bisbibenzyl derivatives. Our previous work on the chemical constituents of *Riccardia multifida* (= *R. multifida* subsp. *decrescens*) resulted in the isolation of two cyclic bisbibenzyls, riccardins A and B, possessing cytotoxic, antimicrobial, and antifungal activity.[1−3] Recently, we isolated four new bisbibenzyl dimers, pusilatins A−D, from the liverwort *Blasia pusilla*.[4−6]

As part of a chemosystematic study and search for biologically active substances of liverworts, we reexamined the chemical constituents of *R. multifida* (L.) S. Gray subsp. *decrescens* (Steph.) Furuki (family Aneuraceae). Here we report the isolation, structure elucidation, and chemical derivatization of a new cyclic bisbibenzyl dimer, pusilatin E (**1**). In addition, the previously known macrocyclic bisbibenzyls riccardin A (**2**),[3] and marchantin I (**3**)[7,8] as well as the norneolignan, egonol 2-methylbutanoate (**4**),[9] were isolated from this same source.

The MeOH extract of *R. multifida* subsp. *decrescens* was subjected to column chromatography on Sephadex LH-20 and Si gel to give pusilatin E (**1**), together with riccardin A (**2**), marchantin I (**3**), and egonol 2-methylbutanoate (**4**).

Pusilatin E (**1**) showed a characteristic deep blue color by TLC after spraying with Godin's reagent[10] and heating at 120 °C. The IR spectrum of **1** indicated the presence of a hydroxyl group (3534 cm^{-1}). The UV spectrum showed strong absorption maxima at 225 (log ϵ 4.85) and 282 nm (log ϵ 4.30). The ^{1}H-NMR spectrum contained signals that integrated for 25 protons comprising benzylic methylenes (δ 2.72−2.97, 8H), a methoxyl group (δ 3.91, 3H), aromatic protons (δ 5.42−7.12, 12H), and phenolic hydroxyl groups (δ 4.82 and 6.23, 2H, interchangeable with D_2O). These signal patterns were closely related to those of riccardin A (**2**), except for the absence of the H-6′ proton signal. Because the FABMS of **1** gave a molecular ion peak at *m*/*z* 874 [M]$^+$, compound **1** was suggested to be a symmetrical dimer of **2**. Furthermore, the proposed structure of **1** was

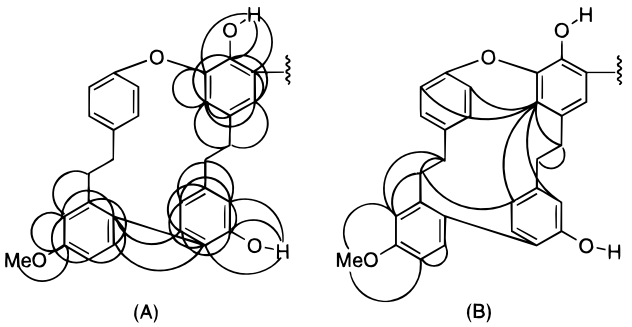
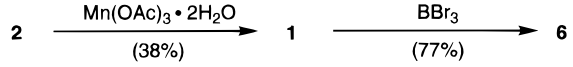


Figure 1. 2D HMBC (A) and NOESY (B) spectra of **1**.

Scheme 1



established by a combination of extensive 2D-NMR experiments employing ^{1}H−^{1}H-COSY, HSQC, HMBC, and NOESY. The ^{1}H−^{1}H-COSY spectrum of **1** revealed the presence of the four independent aromatic rings and an AB system as two doublet signals at δ 5.42 and δ 6.88 (J = 1.9 Hz), assignable to the C-ring protons. As the signal H-6′ was lacking, the coupling pattern showed a difference between **1** and riccardin A (**2**), which exhibited an ABX system for the C-ring protons. Informative cross peaks due to long-range ^{1}H−^{13}C couplings were observed for H-13(H-13″)/C-12′(C-12‴); H-11′(H-11‴)/C-14(C-14″); MeO-11(MeO-11″)/C-11(C-11″); HO-1′(HO-1‴)/C-1′(C-1‴), C-2′(C-2‴), C-6′(C-6‴), and HO-13′(HO-13‴)/C-13′(C-13‴), C-12′(C-12‴), C-14′-(C-14‴) in the HMBC spectrum (Figure 1A). The methoxyl group of **1** was shown to be substituted at C-11(C-11″) on the basis of the NOESY cross peaks observed for H-10(H-10″) and H-12(H-12″) (Figure 1B). Thus, compound **1** was assigned as the C-6′/C-6‴ coupled symmetrical dimer of **2**.

Methylation of **1** with MeI in dry Me_2CO gave the tetramethoxy derivative **5**. The spectral data and chromatographic behavior of this methylated product were identical to those of the pusilatin B (**6**) hexamethyl ether.[6,11]

Chemical derivatization of riccardin A (**2**) by oxidative coupling using $Mn(OAc)_3 \cdot 2H_2O$,[12] provided further evidence for the structure of pusilatin E (**1**) (Scheme 1). Treatment of **2** with 1 equiv of the oxidizing agent in EtOH gave a C-6′/C-6‴ coupled symmetrical dimer **1**

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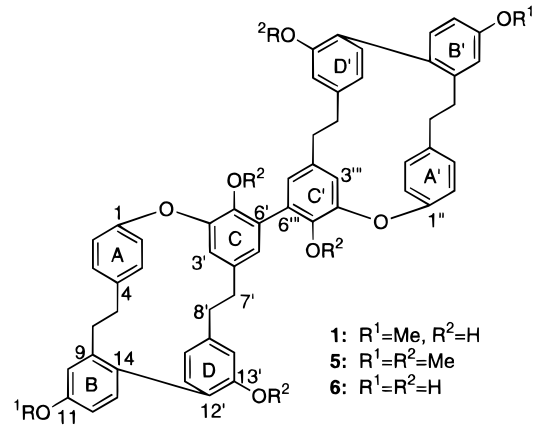
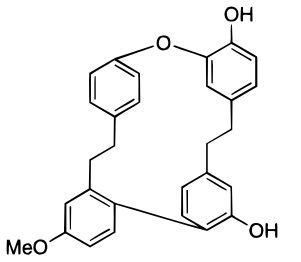
[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1997.

S0163-3864(96)00569-1 CCC: $14.00





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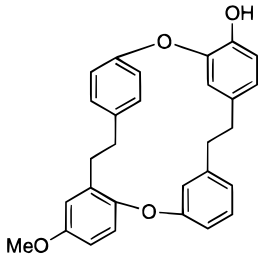

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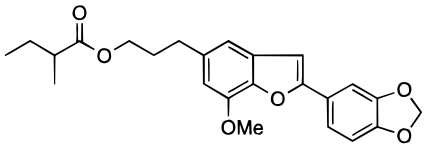

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(yield 38%). Other C−C coupled riccardin A dimers were not detected by TLC, but unidentified polymers derived from **2** were formed. This finding suggests that the C-6′ position of **2** may be a biosynthetically active site in the liverwort. Moreover, the methoxyl groups of **1** were cleaved with boron tribromide to give a demethyl product whose IR, MS, and NMR values were identical to those of the naturally occurring pusilatin B (**6**) (Scheme 1).[5,6,11]

Although the norneolignan egonol 2-methylbutanoate (**4**) has been isolated previously from the immature seeds of *Styrax obassa* (Styracaceae),[9] this is the first record of its isolation from a liverwort. The presence of dimeric bisbibenzyls is very rare, but these compounds are elaborated characteristically in liverworts. Previously, four naturally occurring cyclic bisbibenzyl dimers having the same general structure as **1** have been isolated from *Blasia pusilla*, which belongs to the family Blasiaceae.[4−6] Pusilatin B (**6**) has also been isolated from the axenic cultured liverwort *Ricciocarpos natans* (family Ricciaceae).[11] *B. pusilla*, *R. natans*, and *R. multifida* subsp. *decrescens* are clearly different morphologically, whereas their chemical constituents are similar.

The dimeric bisbibenzyls, pusilatins B (**6**) and C possess DNA polymerase β inhibitory activity.[6] Other bioassays of dimeric bisbibenzyls, including **1**, are now in progress.

Experimental Section

General Experimental Procedures. The mp is uncorrected. The ^{1}H- and ^{13}C-NMR spectra were obtained with a Varian Unity 200 (200 MHz) or a Varian Unity 600 (600 MHz) spectrometer. The IR spectra were measured with a JASCO FT-IR 5300 spectrophotometer. UV spectra were obtained on a Shimadzu UV-300 spectrophotometer. LRFABMS were measured on a JEOL JMS AX-500 spectrometer using *m*-nitrobenzyl alcohol matrix. TLC was carried out on Si gel precoated glass plates with *n*-hexane−EtOAc (1:1 and 4:1) and $CHCl_3$−MeOH (10:1). Detection was with Godin's reagent[10] (just before use, one volume of 1% vanillin solution in EtOH was mixed with one volume of 2% $HClO_4$ in H_2O). For normal-phase column chromatography, Si gel 60 (70−230 mesh, Merck) was used. HPLC purifications were performed with a JASCO pump system using a ChemcoPak Nucleosil 50-5 (7.5 × 250 mm) column.

Plant Material. *R. multifida* (L.) S. Gray subsp. *decrescens* (Steph.) Furuki (Aneuraceae) was collected in May 1996, at Omogo-kei, Ehime, Japan. A voucher specimen (no. 96026) was deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and Isolation. *R. multifida* subsp. *decrescens* (450 g), after being air-dried for 3 days, was extracted twice with MeOH to give a green extract (7.99 g) that was chromatographed on Sephadex LH-20 (MeOH) to afford four fractions (A). Fraction A-4 (1.53 g) was further chromatographed on Sephadex LH-20 using the same eluent to afford an aromatic mixture, which was chromatographed on Si gel (*n*-hexane−EtOAc, gradient) and divided into six fractions (B). Fraction B-3 was purified by means of HPLC (*n*-hexane−EtOAc, 9:1) to yield marchantin I (**3**) (12.1 mg) and egonol 2-methylbutanoate (**4**) (3.2 mg). Fraction B-4 contained riccardin A (**2**) (1.01 g). Fraction B-5 was further purified by HPLC (*n*-hexane−EtOAc, 7:3) to give pusilatin E (**1**) (4.8 mg). The known compounds **2**−**4** were identified through comparison of their spectral data.[3,7−9]

Pusilatin E (1): white powder (EtOAc); mp 195−196 °C; UV (EtOH) λ max (log ϵ) 225 (4.85), 282 (4.30) nm; IR (KBr) ν max 3534 (OH), 3027, 2934, 2857 (CH), 1607, 1505, 1426, 1219, 855, 756 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; FABMS *m/z* 874 [M]$^+$. **Permethyl Pusilatin E (5).** To pusilatin E (**1**, 10.1 mg) in 2 mL of dry Me_2CO was added 2 mL of MeI and 200 mg of dry K_2CO_3. The mixture was kept at reflux for 12 h, and then the reaction mixture was filtered. The solvents were evaporated *in vacuo*, and the residue was purified by means of HPLC (*n*-hexane−EtOAc, 3:2) to afford pusilatin B hexamethyl ether (**5**)[6,11] (7.7 mg).

Coupling Reaction of 2 with $Mn(OAc)_3 \cdot 2H_2O$. To a solution of **2** (195.8 mg, 0.0005 mol) in EtOH (5 mL) was added a solution of $Mn(OAc)_3 \cdot 2H_2O$ (110 mg, 0.0005 mol) in the same solvent (5 mL) under stirring at room temperature. After 1 h, a solution of 1 N HCI (20 mL) was added, and the resulting mixture was extracted with $CHCl_3$ (2 × 50 mL). The combined extracts were dried ($MgSO_4$), the solvent was distilled off, the residue was chromatographed on Si gel (*n*-hexane−EtOAc, gradient) to give pusilatin E (**1**) (73.6 mg; yield 38%), starting material (**2**) (30.0 mg; yield

Table 1. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) Data for Pusilatin E (**1**) in $CDCl_3$[a]

position no.	^{1}H	^{13}C
1/1″		152.7
2/2″	6.84−6.88[b]	122.6[e]
3/3″	6.79−6.90[c]	129.4[f]
4/4″		139.8
5/5″	6.79−6.90[c]	129.4[f]
6/6″	6.84−6.88[b]	122.6[e]
7/7″	2.91 (m)	38.3
	2.97 (m)	
8/8″	2.72 (m)[d]	35.2
	3.08 (m)	
9/9″		143.5
10/10″	7.04 (d, 2.5)	116.3
11/11″		159.9
12/12″	6.89 (dd, 8.5, 2.5)	112.7
13/13″	7.12 (d, 8.5)	132.6
14/14″		128.1
1′/1‴		140.4
2′/2‴		147.3
3′/3‴	5.42 (d, 1.9)	115.5
4′/4‴		133.2
5′/5‴	6.88 (d, 1.9)	124.2
6′/6‴		125.1
7′/7‴	2.72 (m)[d]	37.2
	2.81 (m)	
8′/8‴	2.72 (m)[d]	37.7
9′/9‴		142.0
10′/10‴	6.35 (dd, 7.7, 1.6)	121.7
11′/11‴	6.84 (d, 7.7)	131.5
12′/12‴		124.6
13′/13‴		151.9
14′/14‴	6.47 (d, 1.6)	116.0
1′/1‴−OH	6.23 (s)	
13′/13‴−OH	4.82 (s)	
11/11″−OMe	3.91 (s)	55.4

[a] Chemical shifts from TMS (multiplicity, *J* in Hz). [b−d] Complex multiplet. [e,f] Overlapped signals of two carbons.

15%), and unidentified polymeric compounds (24.1 mg; yield 12%, FABMS: a characteristic fragment ion at *m*/*z* 1309).

Demethylation of 1 with BBr_3. A solution of **1** (22.0 mg) in 2 mL of CH_2Cl_2 was chilled to −78 °C (dry ice−Me_2CO) and boron tribromide (0.2 mL) was add via syringe with stirring under an Ar atmosphere. After being stirred for 3 h at −78 °C, the mixture was slowly warmed to room temperature and quenched with H_2O (10 mL) at 7 °C. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the residue by chromatography on Si gel (*n*-hexane−EtOAc, gradient) afforded pusilatin B (**6**)[5,6,11] (16.4 mg) as a off-white powder.

Acknowledgments. We thank Dr. M. Mizutani, Hattori Botanical Laboratory, Nichinan, Japan, and Dr. T. Furuki, Natural History Museum and Institute, Chiba, Japan, for the identification of *R. multifida* subsp. *decrescens* and Mr. M. Suzuki, Tokushima Bunri University, for helping in the collection of the plant material. Thanks are also due to Dr. M. Tanaka and Miss Y. Okamoto, Tokushima Bunri University, for 600 MHz NMR and mass measurements. This work was partly supported by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan.

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NP9605697