Pergamon

Tetrahedron, Vol. 52, No. 46, pp. 14487-14500, 1996

0040-4020/96 $15.00 + 0.00

PII: S0040-4020(96)00889-7

Phenolic Constituents of the Liverwort: Four Novel Cyclic Bisbibenzyl Dimers from *Blasia pusilla* L.

Tatsuhiko Yoshida, Toshihiro Hashimoto, Shigeru Takaoka, Yukiko Kan[1], Motoo Tori and Yoshinori Asakawa*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan
Fax +81-886-55-3051, E-mail HQL07117@niftyserve.or.jp

John M. Pezzuto, Thitima Pengsuparp and Geoffrey A. Cordell

Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, U.S.A.

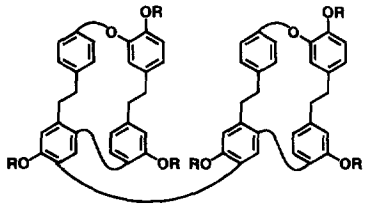
Abstract: Four novel, cyclic, bisbibenzyl dimers (pusilatins A-D), six bibenzyl derivatives, apigenin 7-*O*-β-D-glucoside, shikimic acid and five orsellinic acid derivatives have been isolated from the methanolic extract of the liverwort *Blasia pusilla* L. and their structures characterized by a combination of spectral data, chemical modification and X-ray crystallographic analysis. The previously assigned structure of pusilatin D was revised. Pusilatins B and C showed DNA polymerase β inhibitory activity.

INTRODUCTION

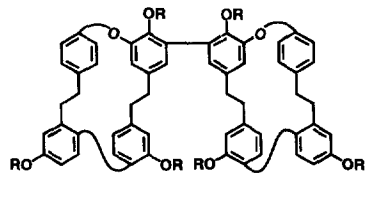
In a continuing search for biologically active natural products with potential as new pharmaceuticals or agrochemicals from the liverwort (Hepaticae), we have reported the distribution of various type of the bioactive cyclic bisbibenzyl derivatives such as the marchantins and the riccardins[2].

Previously, we reported the isolation and structural elucidation of a novel class of bisbibenzyl, the riccardin C dimers, pusilatins A-D (**1-4**) from the Japanese thallic liverwort, *Blasia pusilla* L. (Blasiaceae, Metzgeriales)[3]. On the other hand, several flavonoids and flavonoid glycoside have been isolated from European *B. pusilla*[4], and pusilatin B (6', 6'"-bis-riccardin C) from axenic cultured *Ricciocarpos natans* (L.) Corda (Ricciaceae, Marchatiales) have been reported by Kunz and Becker[5] shortly after our report was published.

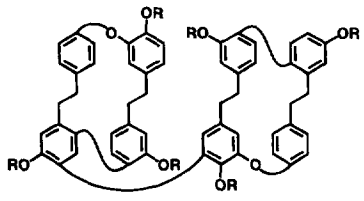
B. pusilla grows on wet soil and contains the symbiotic blue-green alga, *Nostoc* species. Further investigation of the methanolic extract of *B. pusilla* resulted in the isolation of the pusilatin series (**1-4**) and several phenolic compounds (**5-15**). The present paper is concerned with the structural elucidation and biological activities of these isolates and the revision of the structure of pusilatin D.



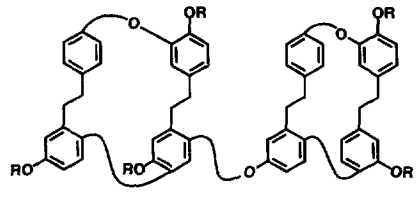
(1) R=H: Pusilatin A
(16) R=Me
(17) R=Ac



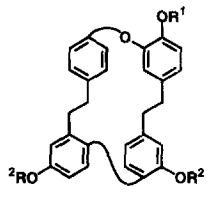
(2) R=H: Pusilatin B
(18) R=Me
(19) R=Ac



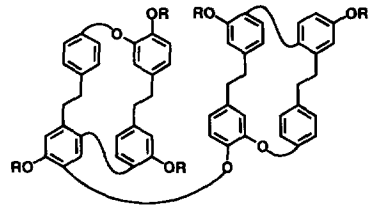
(3) R=H: Pusilatin C
(20) R=Me
(21) R=Ac



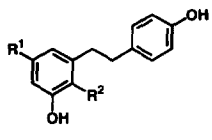
(4a) R=H: Pusilatin D
(22a) R=Ac



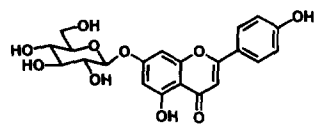
(5) R^1= H, R^2= H: Riccardin C
(6) R^1= Me, R^2= H: Riccardin F
(23) R^1=Me, R^2=Me
(24) R^1=Ac, R^2=Ac



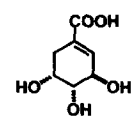
(4b) R=H
(22b) R=Ac



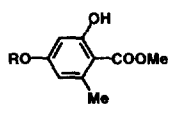
(7) R^1=H, R^2=H: Lunularin
(8) R^1= H, R^2=COOH: Lunularic acid
(9) R^1=OH, R^2=H: Dihydroresveratol



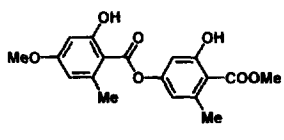
(10) Apigenin 7-*O*-β-D-glucoside



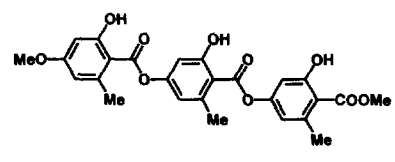
(11) Shikimic acid



(12) R=H: Orsellinic acid methyl ester
(13) R=Me : Everninic acid methyl ester



(14) Methyl evernate



(15) Tenuiorin

Chart 1. Phenolic Constituents of *B. pusilla* L.

RESULTS AND DISCUSSION

B. pusilla was collected in the same location in 1992 and 1993 and each material was extracted with MeOH, respectively. The former extract was subjected to column chromatography on silica gel and Sephadex LH-20 repeatedly to give pusillatins A-D (**1-4**), riccardins C (**5**)[6] and F (**6**)[7], as well as lunularin (**7**)[8], lunularic acid (**8**)[9] and orsellinic acid methyl ester (**12**)[12]. From the latter extract, dihydroresveratol (**9**)[10], apigenin 7-*O*-β-D-glucoside (**10**)[11], shikimic acid (**11**)[17], everninic acid methyl ester (**13**)[13], methyl evernate (**14**)[12, 14] and tenuiorin (**15**)[12, 15] were obtained, together with compounds **1, 3** and **5 - 8** (Chart 1). The structures of compounds **1-6** were preliminarily reported in *Tetrahedron Letters*[3a].

Pusilatin A (**1**)

The IR spectrum of pusilatin A (**1**) indicated the presence of a hydroxyl group (3387 cm^{-1}), an aromatic ring (1611, 1562 cm^{-1}). The ^{13}C NMR spectrum exhibited 28 signals including four benzyl methylene signals (δ 35.7, 37.9, 38.6 and 38.8). The ^1H NMR spectrum indicated the presence of 12 protons on benzene rings at δ 5.37-7.14 ppm and four benzylic methylenes at δ 2.61-2.92 ppm (8H). The decoupling experiments revealed the four independent aromatic rings. The coupling pattern of **1** was very similar to that of riccardin C (**5**) except for the B ring protons. Since **1** gave a parent ion peak in the positive FAB-MS spectrum at *m/z* 869 $[M+Na]^+$ and 846 $[M]^+$, **1** must be a symmetrical dimer of **5**. The methylation and acetylation of **1** gave a hexamethyl ether **16** and a hexa-acetate **17**. Compound **16** showed the NOEs between (i) H-10/10" and 11/11"-OMe, (ii) H-6'/6'" and 1'/1'"-OMe, and (iii) H-14'/14'" and 13'/13'"-OMe (Fig. 1) in the difference NOE spectral experiments. On the basis of the above spectral data, the structure of pusilatin A was suggested to be riccardin C dimer with a C12-C12" phenyl linkage. Furthermore, the structure of **1** was deduced from the analysis of the 2D NMR spectrum of **17**, including ^1H-^1H-COSY, HMQC and HMBC (the assignment of the ^1H and ^{13}C NMR spectra of the acetyl derivative, see Tables 1 and 2) and was finally established by X-ray crystallography of **17** as shown in Fig. 2.

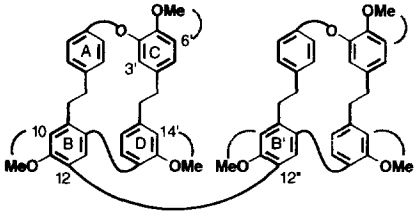


Fig. 1. NOE experiments of **16**.

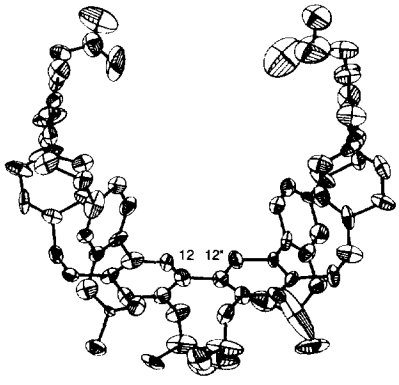


Fig. 2. ORTEP Drawing of **17**.

Pusilatin B (**2**)

The spectral data of pusilatin B (**2**) were almost the same as those of **1**. Methylation of **2** gave a hexamethyl ether **18** which showed the NOEs between (i) H-10/10" and 11/11"-OMe, (ii) H-12/12" and 11/11"-OMe, and (iii) H-14'/14'" and 13'/13'"-OMe (Fig. 3) in the difference NOE spectral experiments. The acetylation of **2** afforded a hexa-acetate **19**. In the HMBC spectrum of **19**, the cross-peaks due to long-range ^1H-^{13}C couplings were observed for H-13 (13")/C-12' (12'") and H-11' (11'")/C-14 (14"), which were diagnostic

for the riccardin type (Fig. 4). The location of each acetyl group was assigned on the basis of ROESY cross-peaks. This compound has been isolated from axenic cultured *Ricciocarpos natans* (L.) Corda[5].

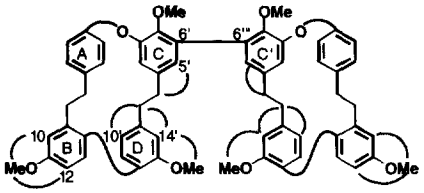


Fig. 3. NOE experiments of **18**.

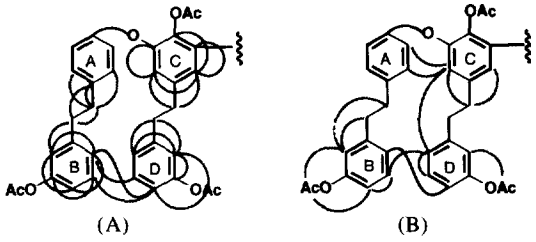


Fig. 4. 2D HMBC (A) and ROESY (B) spectra of **19**.

Pusilatin C (**3**)

In the positive FAB-mass spectrum of **3**, a $[M+Na]^+$ ion peak at *m/z* 869 and a $[M]^+$ ion at *m/z* 846 which was the same molecular weight as seen in compounds **1** and **2**, were observed. The ^1H NMR spectrum of **3** showed exceedingly complex signals at δ 6.67-6.99, two high field proton signals at δ 5.37 and 5.39 (assignable to H-3'/3''' protons) and benzylic methylenes at δ 2.64-2.91 ppm (16H). The ^{13}C NMR spectrum exhibited 56 signals including eight benzyl methylene signals [δ 37.8, 37.89, 37.92 (2 carbons), 38.3, 38.5, 38.7 (2 carbons)]. Therefore, **3** must be an asymmetrical dimer of **5**. Methylation of **3** gave a hexamethyl ether **20** indicating that **3** contained six phenolic hydroxyl groups. Compound **20** showed NOEs between (i) H-10 and 11-OMe, (ii) H-6' and 1'-OMe, (iii) H-14' and 13'-OMe, (iv) H-10'' and 11''-OMe, (v) H-12'' and 11''-OMe, and (vi) H-14''' and 13'''-OMe (Fig. 5) in the 2D-NOESY experiments. The acetylation of **3** afforded a hexa-acetate **21** whose HMBC spectrum contained cross-peaks due to long-range ^1H-^{13}C couplings for H-13/C-12', H-11'/C-14, H-13''/C-12''', H-11'''/C-14'', H-5'''/C-12 and H-13/C-6''' (Fig. 6). Analysis of the ^1H and ^{13}C NMR spectra of **21**, aided by comparison with the data of riccardin C triacetate (**24**) and the extensive 2D-NMR experiments, including ^1H-^1H-COSY, HMQC and HMBC, disclosed that **21** was a dimeric riccardin C (**5**) hexa-acetate linked by a C12-C6''' bond. Thus, the structure of the original pusilatin C is depicted as **3**.

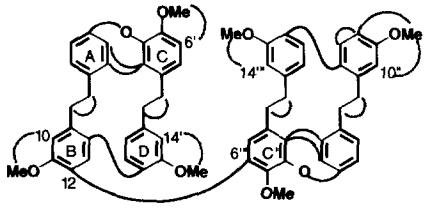


Fig. 5. 2D-NOESY experiments of **20**.

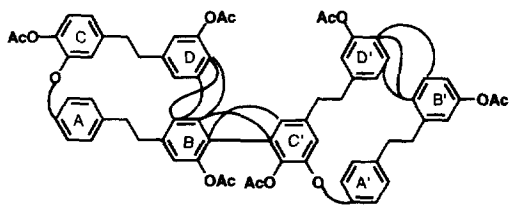


Fig. 6. Key long-range couplings detected in the HMBC spectrum of **21**.

Pusilatin D (**4a**)

Since pusilatin D was not obtained in a pure state, it was acetylated until the absorption band of the hydroxyl group was absent, to give a single product **22** which was purified by chromatography on silica gel (*n*-hexane-EtOAc, gradient). Previously, the structure **22b** was proposed for pusilatin D penta-acetate on the

basis of a comparison of the ^{13}C NMR spectra of **22** with those of related acetyl compounds. Pusilatin D (**4**) was regarded as a dimeric riccardin C (**5**) linked by an ether C12-O-C1''' bond. However, the reduced product of penta-acetate **22** was characterized as **4a** as follows. The UV and IR spectra indicated the absorption maxima at 214 and 285 nm and 3437 cm^{-1}, respectively. FAB-MS spectrometry showed a $[M+Na]^+$ ion at *m/z* 869 and a $[M]^+$ion at *m/z* 846. Analysis of the ^1H and ^{13}C NMR spectra of **4**, aided by comparison with the data of compound **5** (Table 3), suggested that pusilatin D should be depicted as **4a**. The ^1H-^1H COSY and HOHAHA spectrum of **4a** showed the eight independent aromatic rings. Heteronuclear 2D correlation experiments (HSQC and HMBC) were utilized to assign the sp^2 carbon resonances and to obtain structural information regarding the 21 non-protonated carbons. In the HMBC experiment (Fig. 7), three- and two-bond connectivities were observed between the protons of the hydroxy groups and the aromatic carbons. The resonances of C-10' and C-11'' were assigned on the basis of their chemical shifts and long-range correlations to protons (H-11', H-14', H-10'' and H-13'') already identified. Furthermore, the corrected assignments of the penta-acetate **22** are shown in Tables 1 and 2.

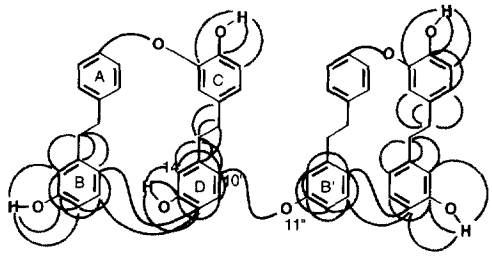


Fig. 7. Key long-range couplings detected in the HMBC spectrum of **4a**.

Other Phenolic compounds (**5-10**) *of Shikimic acid* (**11**) *origin.*

Bibenzyl derivatives **5-8** and the flavonoid glucoside **10** are widely distributed in liverworts[2a]. Dihydroresveratol (**9**) has been isolated from the tubers of *Dioscorea dumentorum* (Dioscoreaceae, yam)[10]. However, this is the first record of its isolation from the liverworts. The isolation and structure of riccardin F (**6**) has been reported previously without any chemical and spectral data. The structure of **6** was further confirmed by chemical correlation (identification of the dimethoxy derivative **23** with riccardin C (**5**) trimethyl ether) and spectroscopic evidence (see Experiment section). Shikimic acid (**11**) is the major component of *B. pusilla*.

Orsellinic acid derivatives (**12-15**)

Orsellinic acid derivatives, including depsides, have been isolated from various lichens[12]. As *B. pusilla* contains the blue-green alga *Nostoc* species, the compounds **12-15** isolated as minor components might originate from this symbiotic *Nostoc* species.

Biological activity of compounds **2, 3, 5** and **6.**

The results of the evaluation of compounds **2**, **3**, **5** and **6** in various polymerase inhibitory activity assays are shown in Table 4. Cytotoxicity ED_{50} values against the nasal epidermoid carcinoma KB and KB-V (drug-resistant KB assessed in the presence or absence of vinblastine) cell lines *in vitro* are revealed in Table 5. Pusilatins B (**2**) and C (**3**) exhibited selective DNA polymerase β inhibitory activity (IC_{50} 13.0 and 5.16 μM) and moderate cytotoxicity.

Table 1. ^{1}H NMR spectral data for compounds 24, 17, 19, 21 and 22 *

Position No.	24†	17††	19†	21§		22§	
2 / (2")	6.78 (brd, 6.0)[a]	6.92 (brd)[g]	6.86 (brs)[i,k]	6.74-6.76[o]		6.73 (brs)[u, w]	6.79 (brs)[v, w]
3 / (3")	6.95 (brd, 6.0)[b,c]	6.69 (brs)[f]	6.98 (brs)[h,l]	6.86 (brs)[p]	6.82 (brs)[p]	6.82-6.86[x]	
5 / (5")	6.62 (brd, 6.0)[b]	6.92 (brd)[g]	6.65 (brs)[h]	6.86 (brs)[p]	6.82 (brs)[p]	6.82-6.86[x]	
6 / (6")	6.69 (brd, 6.0)[a]	6.69 (brs)[f]	6.74 (brs)[i,j]	6.74-6.76[o]		6.72 (brs)[u, w]	6.78 (brs)[v, w]
7 / (7")	2.70 (m)	2.87 (m)[h]	2.72 (m)	2.78 (brs)[q]		2.78-3.11[y, z]	
	2.95 (m)	3.00 (m)	2.97 (m)			2.56 (m)[z]	
8 / (8")	2.58 (m)[d]	2.70 (m)	3.09 (m)	2.91 (brs)[q]		3.22 (m)[z]	
	3.08 (m)	3.11 (m)	2.62 (brs)[m]				
10 / (10")	7.26 (d, 2.4)	7.22 (s)	7.27 (d, 2.4)	7.36 (s)	7.22 (d, 2.4)	7.22 (d, 2.2)	6.87 (d, 2.7)
12 / (12")	7.04 (dd, 8.6, 2.4)	--------	7.05 (dd, 8.3, 2.4)	---------	7.02 (dd, 8.5, 2.4)	6.96 (m)[x]	6.59 (dd, 8.5, 2.7)
13 / (13")	6.96 (d, 8.6)[c]	7.03 (s)	6.98 (d, 8.3)[l]	6.91 (s)	6.97 (d, 8.5)	6.96 (m)[x]	6.93 (d, 8.5)
3' / (3''')	5.33 (d, 2.0)	5.45 (d, 2.0)	5.41 (d, 2.0)	5.56 (d, 2.2)	5.57 (d, 2.2)	5.70 (d, 2.0)	5.54 (d, 2.0)
5' / (5''')	6.92 (dd, 8.1, 2.0)	6.82 (dd, 8.1, 2.0)	6.86 (brs)[k]	6.91 (dd, 8.1, 2.2)	6.82 (d, 2.2)	6.92 (dd, 8.3, 2.0)	6.90 (dd, 8.1, 2.0)
6' / (6''')	7.11 (d, 8.1)	7.01 (d, 8.1)	--------	7.08 (d, 8.1)	--------	7.10 (d, 8.3)	7.07 (d, 8.1)
7' / (7''')	2.58 (m)[d]	2.87 (m)[h]	2.62 (brs)[m]	2.78 (brs)[q]		2.78-3.11[y, z]	
	2.86 (m)[e]		2.91 (brs)[n]				
8' / (8''')	2.58 (m)[d]	2.62 (m)	2.62 (brs)[m]	2.84 (brs)[q]		2.78-3.11[y, z]	
	2.86 (m)[e]	2.87 (m)[h]	2.91 (brs)[n]				
10' / (10''')	6.44 (dd, 7.8, 1.5)	6.44 (brd, 7.8)	6.49 (brd, 7.8)	6.53 (dd, 7.8, 1.7)[r]	6.52 (dd, 7.8, 1.7)[r]	--------	6.50 (dd, 7.8, 1.7)
11' / (11''')	7.02 (d, 7.8)	6.95 (d, 7.8)	7.06 (d, 7.8)	7.02 (d, 7.8)[s]	6.99 (d, 7.8)[s]	6.62 (s)	6.98 (d, 7.8)
14' / (14''')	6.71 (d, 1.5)	6.66 (d, 1.5)	6.74 (brs)[j]	6.74 (d, 1.7)[t]	6.71 (d, 1.7)[t]	6.67 (s)	6.68 (d, 1.7)
-OAc	11; 3H, 2.31 (s)	3H, 2.32 (s)	11/11''; 6H, 2.32 (s)	3H, 2.92 (s)	3H, 2.27 (s)	3H, 2.29 (s)	3H, 2.71 (s)
	1'; 3H, 2.28 (s)	3H, 2.12 (brs)	1'/1'''; 6H, 2.14 (brs)	3H, 2.15 (s)	3H, 2.03 (s)	3H, 2.27 (s)	3H, 2.02 (s)
	13'; 3H, 1.96 (s)	3H, 1.99 (s)	13'/13'''; 6H, 1.98 (s)	3H, 2.00 (s)	3H, 1.94 (s)	3H, 1.84 (s)	

* Chemical shifts from TMS (multiplicity, *J* in Hz).

† 600MHz in DMSO-d_6 at 18 ℃, †† 400MHz in $CDCl_3$ at 27 ℃, § 600MHz in DMSO-d_6 at 100 ℃.

[a, b, h, i, q, u, v, z] May be interchanged in each vertical column, [c, d, e, g, f, j, k, l] Overlapped signals, [o, x, y] Complex multiplet.

[p, r, s, t w] These assignments may be reversed in each row.

Table 2. ^{13}C NMR spectral data for compounds 24, 17, 19, 21 and 22 *

Position No.	**24**†	**17**††	**19**†	**21**§		**22**§	
1 / (1")	152.2	152.9	152.5	152.5[g]	152.4[g]	152.3[r]	152.5[r]
2 / (2")	122.0[a]	128.8[c]	122.3[e]	121.7[h]		121.3[s]	
3 / (3")	129.2[b]	129.2[d]	129.3[f]	128.6[i]		128.7[t]	
4 / (4")	139.6	139.4	139.7	139.2[j]	139.1[j]	139.1	139.1
5 / (5")	129.4[b]	129.2[d]	129.5[f]	128.6[i]		121.3[s]	
6 / (6")	122.3[a]	128.8[c]	122.0[e]	121.7[h]		128.7[t]	
7 / (7")	37.4	38.0	37.4	35.9[k]	35.8[k]	34.7	35.5
8 / (8")	34.6	35.2	34.7	34.3[k]	34.2[k]	34.1[u]	34.4[u]
9 / (9")	142.5	148.1	142.5	141.9	142.2	142.5[v]	142.0[v]
10 / (10")	123.1	124.0	123.1	123.6	122.2	122.7	118.7
11 / (11")	150.3	142.1	150.3	147.3	150.2[l]	149.8[w]	156.1
12 / (12")	119.6	127.8	119.6	126.8	118.5	118.5	115.2
13 / (13")	131.7	132.3	131.8	132.5	131.3[m]	131.2[y]	131.2[y]
14 / (14")	134.0	134.6	134.0	133.5	133.4	132.6	130.6[z]
1' / (1''')	137.3	137.9	135.1	137.3	135.2	137.3	137.4
2' / (2''')	150.1	150.6	150.5	150.0[l]	149.8[l]	150.0[w]	150.2[w]
3' / (3''')	117.1	117.7	116.9	116.9	116.6	116.4	116.9
4' / (4''')	139.3	139.6	138.7	138.9	138.2	139.1	138.9
5' / (5''')	122.1	121.9	123.5	121.6	123.1	121.7	121.7
6' / (6''')	123.2	122.9	131.2	122.5	130.8	122.5[c]	
7' / (7''')	36.5	37.2	36.6	36.8	36.7[k]	36.8[u]	36.8[u]
8' / (8''')	36.4	37.1	36.6	35.5	35.5	35.7[u]	30.3[u]
9' / (9''')	141.1	147.4	141.0	140.8[n]	140.5[n]	131.0[z]	140.4
10' / (10''')	127.0	127.4	127.0	126.1[o]	125.9[o]	150.8	126.0
11' / (11''')	132.1	132.3	132.1	131.2[m]	131.0[m]	121.0	131.3[y]
12' / (12''')	130.6	130.6	130.7	129.9	130.2	131.7	130.4
13' / (13''')	147.0	141.5	147.0	146.9	146.9	142.7	147.0
14' / (14''')	122.5	122.5	122.5	121.9[p]	121.8[p]	124.8	121.5
-OCO$\underline{C}$H$_3$	21.0 (11)	20.8	21.0 (11/11")	19.2	20.2	20.2	19.8
	20.5 (1')	20.7	20.3 (1'/1''')	19.7[q]		19.7[x]	19.5
	20.5 (13')	20.7	20.5 (13'/13''')	19.9			
-O$\underline{C}$OCH$_3$	169.2 (11)	169.1	169.2 (11/11")	168.2	167.9	168.2	168.1
	168.7 (1')	169.1	168.2 (1'/1''')	167.8	167.7	167.8	167.8
	168.6(13')	168.8	168.6 (13'/13''')	167.7	167.2	167.5	

* Chemical shifts from TMS (multiplicity, J in Hz).

† 150MHz in DMSO-d_6 at 18 ℃, †† 100MHz in $CDCl_3$ at 27 ℃, § 150MHz in DMSO-d_6 at 100 ℃.

[a, b, e, f] May be interchanged in each vertical column.

[c, d, x] Overlapped signals of 2 carbons, [q] Overlapped signals of 3 carbons.

[h, i, s, t] Overlapped signals of 4 carbons.

[g, j, n, o, p, r, v, y, w] These assignments may be reversed in each row.

[k, l, m, u, w, z] Assignments may be interchangeable.

Table 3. ^{1}H (600MHz) and ^{13}C (150MHz) data for riccardin C (5) and pusilatin D (4) [a]

Position No.	Riccardin C (5)*		Pusilatin D (4)†			
	^{1}H	^{13}C	^{1}H		^{13}C	
1 / (1'')	-----	152.5	-----	-----	154.6[l]	154.2[l]
2 / (2'')	6.72-6.80[b]	122.3[e]	6.71 (m)[g, h]	6.92 (m)[h, i]	123.0[m]	
3 / (3'')	6.87 (brs)[c]	129.2[f]	6.84 (m)[j]	7.07 (m)[j]	130.3[n]	
4 / (4'')	-----	139.8	-----	-----	140.9[o]	140.7[o]
5 / (5'')	6.87 (brs)[c]	129.2[f]	6.84 (m)[j]	7.07 (m)[j]	130.3[n]	
6 / (6'')	6.72-6.80[b]	122.3[e]	6.71 (m)[g, h]	6.71 (m)[g, h]	123.0[m]	
7 / (7'')	2.88 (m)	38.1	2.52-3.04[k]		38.8[p]	39.1[p]
8 / (8'')	2.95 (m) 2.23-2.75[d] 3.03 (m)	35.0	2.52-3.04[k]		35.9	36.0
9 / (9'')	-----	143.7	-----	-----	143.8	144.7
10 / (10'')	6.96 (d, 2.9)	117.5	6.92 (d, 2.4)[i]	6.85 (d, 2.7)	117.9	118.4
11 / (11'')	-----	155.9	-----	-----	157.9	159.7
12 / (12'')	6.79 (dd, 8.6, 2.9)	114.3	6.71 (m)[g]	6.55 (dd, 8.6, 2.7)	113.9	114.9
13 / (13'')	7.03 (d, 8.6)	132.8	7.14 (d, 8.5)	7.07 (d, 8.6)	133.8	133.7
14 / (14'')	-----	128.2	-----	-----	129.1	132.9
1' / (1''')	-----	143.7	-----	-----	145.5	145.3
2' / (2''')	-----	146.3	-----	-----	148.2	148.0
3' / (3''')	5.35 (d, 2.0)	116.0	5.62 (d, 2.0)	5.39 (d, 2.0)	118.1	117.7
4' / (4''')	-----	133.1	-----	-----	133.5[q]	133.3[q]
5' / (5''')	6.73 (dd, 8.1, 2.0)	122.1	6.73 (brd, 8.1)[g]	6.73 (brd, 7.8)[g]	122.9	122.8
6' / (6''')	6.92 (d, 8.1)	114.9	6.86 (d, 8.1)	6.83 (d, 7.8)	116.8	116.6
7' / (7''')	2.23-2.75[d]	37.1	2.52-3.04[k]		35.4	37.9
8' / (8''')	2.23-2.75[d]	37.6	2.37 (brt, 10.7) 3.17 (ddd, 12.5, 10.7, 2.8)	2.52-3.04[k]	33.3	38.6
9' / (9''')	-----	141.9	-----	-----	132.7	142.1
10' / (10''')	6.23 (dd, 7.8, 1.7)	121.7	-----	6.16 (dd, 7.7, 1.7)	146.7	121.6
11' / (11''')	6.77 (d, 7.8)	131.4	6.62 (s)	6.82 (d, 7.7)	124.9	133.1
12' / (12''')	-----	124.4	-----	-----	127.9	126.4
13' / (13''')	-----	151.8	-----	-----	151.2	154.4
14' / (14''')	6.39 (d, 1.7)	116.0	6.21 (s)	6.39 (d, 1.7)	120.1	117.8
-OH	4.84 (brs) 5.22 (brs) 5.64 (brs)		8.28 (s, 11) 7.69 (s, 1') 7.63 (s, 13')	----- 7.64 (s, 1''') 7.68 (s, 13''')		

* in $CDCl_3$ at 25 ℃, † in acetone-d_6 at 35 ℃.

[a] Chemical shifts from TMS (multiplicity, *J* in Hz).

[b, d, k] Complex multiplet.

[c, g, i] Overlapped signals.

[e, f] Overlapped signals of 2 carbons.

[m, n] Overlapped signals of 4 carbons.

[h, j, l, o, p, q] These assignments may be reversed in each row.

Table 4. Polymerase assay data for compounds 2, 3, 5 and 6 *

No.	HIV-1 RT	HIV-2 RT	Mutant RT†	AMV RT	DNA pol. β	RNA pol.
2	IA	IA	IA	IA	13.0	IA
3	IA	IA	IA	IA	5.16	IA
5	WA	IA	IA	265.7	25.9	IA
6	WA	IA	IA	IA	38.5	IA

* IC_{50}, μM, IA=inactive, WA=weakly active.
† TIBO-resistant HIV-1 reverse transcriptase.

Table 5. Cytotoxicity data *

No.	KB	KB-V (+VLB)	KB-V (-VLB)
2	13.1	15.3	11.9
3	13.0	7.1	11.7
5	13.4	13.6	16.4
6	>20	>20	>20

* ED_{50}, μg ml^{-1}.

EXPERIMENTAL

General Methods. Thin-layer chromatography (TLC) was carried out on silica gel precoated glass plates (silica gel 60 F_{254}, Merck) with *n*-hexane-AcOEt (4:1, 1:1) and $CHCl_3$-MeOH (10:1, 5:1). For normal phase column chromatography (CC), silica gel 60 (70-230 mesh, Merck) was used. The mix. of $CHCl_3$-MeOH (1:1) was used for CC on Sephadex LH-20 as solvent. HPLC purifications were performed by JASCO pump system using ChemcoPak Nucleosil 50-5 (7.5x250mm). Lobar Rp-18 (310x25mm, Merck) was used for MPLC purifications.

Spectral data. The ^1H and ^{13}C NMR spectra were taken with a Varian Unity 200 (200MHz), a JEOL JNM GX 400 (400Mz) or a Varian Unity 600 (600MHz). ^{13}C multiplicities were established by the DEPT pulse sequence. The IR spectra were measured with a JASCO FT/IR-5300 spectrophotometer. UV spectra were obtained on a Shimadzu UV-300 spectrophotometer. Low-resolution FAB mass spectra were measured on a JEOL JMS AX-500 spectrometer using glycerol or *m*-nitrobenzyl alcohol matrix and Xe ionizing gas. EI mass were measured at 70 eV.

Plant Materials. *Blasia pusilla* L. was collected in 1992 and 1993 at Sanagouchi, Tokushima, Japan and identified by Dr. M. Mizutani. The voucher specimens were deposited in the herbarium at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and Isolation.

B. pusilla L. (1.25 kg) collected in 1992 was mechanically ground and the resulting powder was extracted with MeOH for 4 weeks to give a green extract (98.5 g) after being filtered and the solvent evaporated. This extract was partitioned between EtOAc and water. The organic layer was concentrated *in vacuo* to afford the viscous oil (30.5 g) which was chromatographed on Sephadex LH-20 and divided into 5 fractions (A). Fraction A-5 (3.03 g) was further divided by CC on silica gel ($CHCl_3$-MeOH, gradient) to give 14 fractions (B). Fraction B-2 was further chromatographed on Sephadex LH-20 and CC of silica gel (*n*-hexane-EtOAc, gradient) to yield orsellinic acid methyl ester **(12)** (12.5 mg). Fraction B-3 contained riccardin F **(6)** (183.1 mg). Fraction B-4 included riccardin C **(6)** (666.9 mg). Fraction B-4 (77.1 mg) was acetylated with Ac_2O/py to give a mixture of acetates. The B-4 acetate mix. was purified by CC on silica gel (*n*-hexane-EtOAc, gradient) to afford compound **17** (17.7 mg) and the diacetate of **7** (12.9 mg). Fraction B-6 (115.3 mg) was

acetylated in the same manner as described above to yield a mixture of acetates which was purified by CC on silica gel (*n*-hexane-EtOAc, gradient) to give compounds **17** (28.9 mg), **21**(49.4 mg) and **22** (28.0 mg). Fraction B-7 contained pusilatin C (**4**) (246.2 mg). Fraction B-8 (320.3 mg) was further divided into 6 fractions (C) by CC on Sephadex LH-20. Fraction C1-3 contained pusilatin B (**2**) (64.8 mg). Fraction C-4 (41.7 mg) was methylated with MeI/K_2CO_3 to give a mixture of methyl ethers which was purified by CC on Sephadex LH-20 to give compound **20** (16.3 mg) and the trimethyl ether of **8** (13.3 mg).

B. pusilla L. (1.18 kg) collected in 1993 was extracted with MeOH for 4 weeks to give a green extract (31.0 g) after filtration and evaporation. The methanolic extract was chromatographed on Sephadex LH-20 to give a fraction of phenolic constituents (6.17 g). This fraction was further separated by CC on silica gel ($CHCl_3$-MeOH, gradient) to give 14 fractions (D). Fraction D-1 contained a precipitate which was filtered off through a short column packed with cotton and the solvent evaporated to give tenuiorin (**15**) (9.4 mg). The mother liquor was evaporated and chromatographed on silica gel (*n*-hexane-EtOAc, gradient) to yield everninic acid methyl ester (**13**) (5.2 mg) and methyl evernate (**14**) (0.8 mg). Fraction D-2 was subjected to CC on silica gel (*n*-hexane-EtOAc, gradient) to afford **5** (212.1mg) and **6** (384.3 mg). Fraction D-3 (356.9 mg) was subjected to CC on Sephadex LH-20 and reverse phase MPLC (MeOH:H_2O = 9:1) to give lunularin (**7**) (44.5 mg), pusilatin A (**1**) (7.7 mg), pusilatin C (**3**) (36.1 mg) and riccardin C (**5**) (44.2 mg). Fraction D-4 was further separated by CC on Sephadex LH-20 to give lunularic acid (**8**) (5.7 mg) and dihydroresveratol (**9**) (3.3 mg). Fraction D-5 was recrystallized from MeOH to yield apigenin 7-*O*-β-D-glucoside (**10**) (24.4 mg) and shikimic acid (**11**) (908.8 mg).

The known compounds **5**, **7-9** and **11-15** were identified through comparison of their spectral data with those reported in refs. 6-15.

Pusilatin A **(1).** Light yellow powder, ^{1}H-NMR (in acetone-d_6, 200MHz) δ: 2.61, 2.92 (8H, brs), 5.37 (1H, d, *J*=1.8 Hz), 6.14 (1H, dd, *J*=7.8, 1.5 Hz), 6.34 (1H, d, *J*=1.5 Hz), 6.71 (2H, m), 6.75 (1H, brs), 6.82 (1H, d, *J*=8.0Hz), 6.85 (1H, d, *J*=7.8Hz), 6.93* (2H, m), 7.11 (1H, s), 7.14 (1H, s), 7.69 (1H, s, OH), 8.01 (1H, s, OH), 8.56(1H, brs, OH) (*overlapped signals). ^{13}C-NMR (in acetone-d_6, 50MHz) δ : 35.7(t), 37.9(t), 38.6(t), 38.8(t), 116.6(d), 117.1(d), 117.6(d), 119.0(d), 121.4(d), 122.7(d), 122.9*(d), 124.8(s), 126.5(s), 130.3*(d), 133.1(d), 131.7(s), 133.3(s), 135.6(d), 140.8(s), 142.0(s), 143.8(s), 145.3(s), 148.0(s), 154.12(s), 154.18(s), 154.4(s) (*overlapped signals of 2 carbons). FT-IR $\upsilon^{KBr}cm^{-1}$: 3387, 1611, 1562, 1504, 1223, 1111. Positive FAB-MS *m/z*: 869 $[M+Na]^+$, 846 $[M]^+$. UV λ_{max} (EtOH) nm (log ε): 212 (4.62), 240 (4.49), 285 (4.10).

Pusilatin A hexamethyl ether **(16).** To pusilatin A (**1**, 7.7 mg) in dry acetone (2 ml) was added MeI (2 ml) and dry K_2CO_3 (200 mg). The mixture was kept at reflux for 12 h, and the reaction mixture filtered. The solvents were evaporated *in vacuo*, and the residue was purified by means of HPLC (*n*-hexane-EtOAc = 3:2) to afford **16** (6.7 mg). Off-white powder, ^{1}H-NMR (in $CDCl_3$, 200MHz) δ: 2.61 (2H, m), 2.80 (3H, m), 3.23 (1H, m) (methylenes), 3.67 (3H, s, 13'/13'''-OMe), 3.88 (3H, s, 11/11''-OMe), 3.94 (3H, s, 1'/1'''-OMe), 5.36 (1H, d, *J*=1.8 Hz, 3'/3'''-H), 6.20 (1H, brd, *J*=7.5 Hz, 10'/10'''-H), 6.44 (1H, brs, 14'/14'''-H) *ca.* 6.72* (3H, brs), *ca.* 6.85* (1H, brs) (A/A' ring-H), 6.77 (1H, dd, *J*=8.2, 1.8 Hz, 5'/5'''-H), 6.83 (1H, d, *J*=7.5 Hz, 11'/11'''-H), 6.84 (1H, d, *J*=8.2 Hz, 6'/6'''-H), 6.97 (1H, s, 10/10''-H), 7.09 (1H, s, 13/13''-H) (*overlapped signals). ^{13}C-NMR (in $CDCl_3$, 50MHz) δ: 35.7(t), 37.3(t), 38.1(t), 38.2(t), 55.2(q), 55.8(q), 56.1(q), 110.9(d), 111.6(d), 112.4(d), 116.6(d), 121.4(d), 121.7(d), 122.3*(d), 125.2(s), 127.8(s), 129.3(d), 129.6(d), 132.7(s), 133.8(d), 135.0(d), 139.8(s), 141.0(s), 146.8(s), 148.6(s), 152.7(s), 156.0(s), 156.7(s). (*overlapped signals of 2 carbons). FT-IR $\upsilon^{KBr}cm^{-1}$: 2932, 2855, 1609, 1507, 1262, 1231, 1128, 1040. Positive FAB-MS *m/z*: 930 $[M]^+$. UV λ_{max} (dioxane) nm (log ε): 242 (4.67), 282 (4.33).

***Pusilatin A hexa-acetate* (17).** Fraction B-6, including pusilatins A (**1**), C (**3**) and D (**4**) (115.3 mg), was dissolved in pyridine (4 ml) and Ac_2O (4 ml). The mixture was allowed to stand overnight at room temp. Usual work-up gave a product, which was chromatographed on silica gel (*n*-hexane-EtOAc, gradient) to afford **17** (49.8 mg) together with **21** (49.4 mg) and **22** (28.0 mg). Compound **17**: White powder, m.p.: 293.0-293.5°. ^{1}H- and ^{13}C-NMR δ: see Tables 1 and 2. FT-IR υ^{KBr}cm^{-1}: 3399, 1616, 1504, 1423, 1336, 1223, 1018. Positive FAB-MS *m/z*: 869 $[M+Na]^+$, 846 $[M]^+$. UV λ_{max} (dioxane) nm (log ε): 227 (4.12), 285 (2.81).

***Crystal data for* 17.** Crystal dimensions = 0.10 x 0.10 x 0.10 mm, monoclinic, space group *Cc* (no. 9) with *a*=30.483 (6)Å, *b*=14.330 (3), *c*=17.106 (3)Å, β=122.86(1)°, *V*=6277(2)Å^3, *Z*=4, *F*(000)=2312, D_{calc} = 1.16g cm^{-3}, and μ(Cu Kα)=5.86cm^{-1} by Mac Science MXC 18 diffractometer at room temperature. Final *R* and R_w were 0.102, 0.111 for 3759 reflections. The supplementary materials have been deposited at the Cambridge Crystallographic Data Centre.

***Pusilatin B* (2).** White powder, m.p.: 293.0-293.5°. ^{1}H-NMR (in acetone-d_6, 200MHz) δ: 2.72, 2.94, 3.00 (8H, brs), 5.40 (1H, d, *J*=2.0 Hz), 6.25 (1H, dd, *J*=7.8, 1.6 Hz), 6.45 (1H, d, *J*=1.6 Hz), 6.74 (1H, dd, *J*=8.4, 2.6 Hz), 6.80 (1H, d, *J*=7.8Hz), *ca.* 6.85* (4H, m), 6.91 (1H, d, *J*=2.0Hz), 6.95 (1H, d, *J*=2.6Hz), 6.99 (1H, d, *J*=8.4 Hz), 7.66, 7.87, 8.32 (each 1H, brs, OH) (*overlapped signals). ^{13}C-NMR (in acetone-d_6, 50MHz) δ: 36.2(t), 38.3(t), 38.8(t), 39.0(t) 114.2(d), 116.57(d), 116.60(d), 117.2(d), 117.6(d), 121.72(d), 121.75(d), 123.3(d), 125.3(d), 127.0(s), 127.6(s), 130.5(d), 130.6(s), 133.3(d), 133.4(s), 133.7(d), 141.1(s), 142.0(s), 142.5(s), 1445(s), 149.0(s), 154.2(s), 154.7(s), 158.0(s). FT-IR υ^{KBr}cm^{-1}: 3399, 1616, 1504, 1423, 1336, 1223, 1018. Positive FAB-MS *m/z*: 869 $[M+Na]^+$, 846 $[M]^+$. UV λ_{max} (EtOH) nm (log ε): 227 (4.12), 285 (2.81).

***Pusilatin B hexamethyl ether* (18).** Fraction C-4 including pusilatin B (**2**, 41.7 mg) was methylated in the same manner as described above to give **18** (16.3 mg) which was purified by CC on Sephadex LH-20 ($CHCl_3$-MeOH = 1:1). Off-white powder, ^{1}H-NMR (in $CDCl_3$, 200MHz) δ: 2.72, 2.91, 3.05 (8H, m, methylenes), 3.69 (3H, s, 13'/13'''-OMe), 3.85 (3H, s, 1'/1'''-OMe), 3.89 (3H, s, 11/11''-OMe), 5.46 (1H, d, *J*=2.0 Hz, 3'/3'''-H), 6.30 (1H, dd, *J*=7.7, 1.5 Hz, 10'/10'''-H), 6.46 (1H, d, *J*=1.5 Hz, 14'/14'''-H), *ca.* 6.77* (4H, m, A/A' ring-H), 6.83 (1H, dd, *J*=8.5, 2.5Hz, 12/12''-H), 6.86 (1H, d, *J*=2.0Hz, 5'/5'''-H), 6.87 (1H, d, *J*=7.7Hz, 11'/11'''-H), 6.98 (1H, d, *J*=2.5Hz, 10/10''-H), 7.08(1H, *J*=8.5Hz, 13/13''-H) (*overlapped signals). ^{13}C-NMR (in $CDCl_3$, 50MHz) δ: 35.6(t), 37.4(t), 37.9(t), 38.2(t), 55.2*(q), 61.0(q), 111.2(d), 111.4(d), 115.4(d), 117.2(d), 121.7(d), 122.2*(d), 124.3(d), 127.6(s), 129.4*(d), 130.9(s), 132.4(d), 132.5(d), 132.7(s), 136.0(s), 139.7(s), 141.2(s), 143.3(s), 144.9(s), 152.1(s), 153.2(s), 156.0(s), 159.1(s) (*overlapped signals of 2 carbons). Positive FAB-MS *m/z*: 969 $[M+K]^+$, 930 $[M]^+$. FT-IR υ^{KBr}cm^{-1}: 2936, 2860, 1605, 1505, 1415, 1222, 1041. UV λ_{max} (dioxane) nm (log ε): 241 (4.61), 280 (4.22).

***Pusilatin B hexa-acetate* (19).** Pusilatin B(**2**, 21.5 mg) was acetylated in the same manner as described above to yield **19** (20.4 mg) which was purified by HPLC (*n*-hexane-EtOAc = 3:2). Light-yellow powder, ^{1}H- and ^{13}C-NMR δ: see Tables 1 and 2. FT-IR υ^{KBr}cm^{-1}: 1765, 1587, 1505, 1419, 1195, 1014. Positive FAB-MS *m/z*: 1137 $[M+K]^+$, 1099 $[M+H]^+$. UV λ_{max} (dioxane) nm (log ε): 240 (4.44).

***Pusilatin C* (3).** Light yellow powder, ^{1}H-NMR (in acetone-d_6, 200MHz) δ: 2.64 (8H, brs), 2.91 (8H, brd, *J*=12.1 Hz) (methylenes), 5.37, 5.39 (each 1H, d, *J*=1.8Hz), 6.19 (2H, brd, *J*=7.5Hz), 6.67-6.99* (18H, m), 7.12, 7.20 (each 1H, s), 7.62, 7.75, 7.98 (each 1H, s, OH), 8.07, 8.32, 8.45 (each 1H, brs, OH) (*overlapped signals). ^{13}C-NMR (in acetone-d_6, 50MHz) δ: 37.8(t), 37.89(t), 37.92*(t), 38.3(t), 38.5(t), 38.7*(t), 113.9(d), 116.3(d), 116.5(d), 116.8(d), 117.0(d), 117.3(d), 119.4(d), 121.4*(d), 122.7(d), 122.9**(d), 125.0(d), 125.0(s),

126.4(s), 126.6(s), 127.0(s), 130.2**(d), 130.3(d), 131.4(s), 133.02(d), 133.06(d), 133.2(d), 133.4(s), 133.8(s), 135.2(d), 140.7(s), 140.9(s), 141.4(s), 141.6(s), 141.8(s), 143.8(s), 144.1(s), 145.1(s), 147.9(s), 148.3(s), 153.7(s), 154 0(s), 154.26(s), 154.32(s), 154.5(s), 157.6(s) (*overlapped signals of 2 carbons, **overlapped signals of 4 carbons). FT-IR $\upsilon^{KBr}cm^{-1}$: 3433, 1608, 1505, 1433, 1221, 1113, 816. Positive FAB-MS *m/z*: 869 $[M+Na]^+$, 846 $[M]^+$. UV λ_{max} (EtOH) nm (log ε): 215 (4.72), 285 (4.11).

***Pusilatin C hexamethyl ether* (20).** A mixture including pusilatin C (**3**, 30.2 mg) was treated in the same manner as described above to afford **20** (24.6 mg) which was purified by CC on silica gel (*n*-hexane-EtOAc gradient) and Sephadex LH-20 ($CHCl_3$-MeOH = 1:1). White powder, m.p.: 144.5-150.0°. ^{1}H-NMR (in $CDCl_3$, 600MHz) δ: 2.62, 2.71 (each 3H, m), 2.85 (5H, m), 2.94 (2H, m), 3.02, 3.08, 3.19 (each 1H, m) (methylenes), 3.66, 3.71 (each 3H, s, 13'/13'''-OMe), 3.83 (3H, brs, 1'''-OMe), 3.87 (3H, s, 11''-OMe), 3.93 (3H, s, 11-OMe), 3.94 (3H, s, 1'-OMe), 5.39 (1H, d, *J*=1.9Hz, 3'''-H), 5.40 (1H, d, *J*=2.2Hz, 3'-H), 6.22 (1H, brd, *J*=7.4Hz), *ca.* 6.32 (1H, m) (10'/10'''-H), 6.43, 6.47 (each 1H, brs, 14'''/14'-H), 6.75, *ca.* 6.89, 6.94 (8H, m, A/A' ring-H), 6.78 (1H, dd, *J*=8.3, 2.2Hz, 5'-H), 6.79 (1H, d, *J*=1.9Hz, 5'''-H), 6.81 (1H, dd, *J*=8.5, 2.7Hz, 12''-H), *c.a.* 6.83 (1H, m), 6.90 (1H, d, *J*=7.4Hz) (11'''/11'-H), 6.88 (1H, d, *J*=8.3Hz, 6'-H), 7.02 (1H, s, 10-H), 7.05 (1H, brd, *J*=8.5Hz, 13''-H), 7.11 (1H, s, 13-H). ^{13}C-NMR (in $CDCl_3$, 50MHz) δ: 35.6(t), 35.8(t), 37.3*(t), 37.7(t), 38.1(t), 38.18(t), 38.25(t), 55.18(q), 55.21(q), 55.3(q), 55.7(q), 56.1(q), 60.9(q), 111.1(d), 111.4(d), 111.7(d), 112.0(d), 115.3(d), 116.6(d), 116.8(d), 121.4(d), 121.6(d), 121.8(d), 122.2*(d), 122.3*(d), 124.5(d), 125.3(s), 127.5(s), 127.6(s), 129.3**(d), 129.4(d), 130.5(s), 130.9(s), 132.3(d), 132.5(d), 132.55(d), 132.64(s), 133.8(s), 134.5(d), 135.9(s), 139.6(s), 139.7(s), 141.18(s), 141.23(s), 142.1(s), 143.3(s), 145.1(s), 146.9(s), 148.6(s), 152.0(s), 152.7(s), 153.1(s), 155.9(s), 156.0(s), 156.4(s), 159.1(s) (*overlapped signals of 2 carbons, **overlapped signals of 4 carbons). Positive FAB-MS *m/z*: 969 $[M+K]^+$, 930 $[M]^+$. FT-IR $\upsilon^{KBr}cm^{-1}$: 2932, 2855, 1607, 1505, 1420, 1219, 1042. UV λ_{max} (dioxane) nm (log ε): 244 (4.56), 282 (4.25).

***Pusilatin C hexa-acetate* (21).** A mixture including pusilatin C (**3**, 41.8 mg) was treated in the same manner as described above to yield **21** (15.0 mg) which was purified by CC on silica gel (*n*-hexane-EtOAc gradient). White powder, ^{1}H- and ^{13}C-NMR δ: see Tables 1 and 2. Off-white powder, FT-IR $\upsilon^{KBr}cm^{-1}$: 1765, 1589, 1505, 1424, 1370, 1267, 1198, 1015. Positive FAB-MS *m/z*: 1121 $[M+Na]^+$, 1099 $[M+H]^+$. UV λ_{max} (dioxane) nm (log ε): 245 (4.52).

***Pusilatin D penta-acetate* (22).** Light yellow powder, ^{1}H- and ^{13}C-NMR δ: see Tables 1 and 2. FT-IR $\upsilon^{KBr}cm^{-1}$: 1763, 1595, 1505, 1422, 1370, 1267, 1201, 1115. Positive FAB-MS *m/z*: 1095 $[M+K]^+$, 1079 $[M+Na]^+$, 1057 $[M+H]^+$. UV λ_{max} (dioxane) nm (log ε): 248 (4.19).

***Reduction of* 22 *with Lithium Aluminum Hydride*.** To an ice-cooled suspension of $LiAlH_4$ (15.2 mg) in dry ether (6 ml) was added **22** (28.0 mg) in THF (5 ml) over 10 min. with stirring. The mixture was stirred overnight at room temp., and then excess reagent was decomposed by careful addition of water. The precipitate formed was dissolved by 1N HCl, and the solution was extracted with EtOAc. The combined extracts were washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel (*n*-hexane-EtOAc gradient) to afford pusilatin D (**4**) (5.4 mg). Light yellow powder, ^{1}H- and ^{13}C-NMR δ: see Table 3. FT-IR $\upsilon^{KBr}cm^{-1}$: 3437, 1605, 1506, 1433, 1271, 1227, 1113. Positive FAB-MS *m/z*: 869 $[M+Na]^+$, 846 $[M]^+$. UV λ_{max} (EtOH) nm (log ε): 214 (4.66), 285 (3.91).

***Riccarin F* (6).** Light yellow powder, ^{1}H-NMR (in $CDCl_3$, 200MHz) δ: *ca.* 2.80 (8H, m, methylenes), 3.94 (3H, s, 1'-OMe), 5.40 (1H, d, *J*=1.8 Hz, 3'-H), 6.20 (1H, dd, *J*=7.9, 1.5 Hz, 10'-H), 6.40 (1H, d, 14'-H) *ca.*

6.72* (1H, m, 5'-H), *ca.* 6.75* (1H, m, 12-H), 6.75* (1H, d, *J*=7.9 Hz, 11'-H), *ca.* 6.76* (4H, m, A ring-H), 6.89 (1H, d, *J*=8.2 Hz, 6'-H), 6.92 (1H, d, *J*=2.7 Hz, 10-H) 6.97 (1H, d, *J*=8.4 Hz, 13-H) (*overlapped signals). NOEs were observed between the following sets of protons: 1'-OMe→6'-H, methylen protons→ 10-H, 3'-H, 5'-H, 10'-H, 14'-H (in $CDCl_3$, 400MHz). ^{13}C-NMR (in $CDCl_3$, 50MHz) δ: 34.9(t), 37.0(t), 37.6(t), 38.0(t), 56.0(q), 111.8(d), 114.3(d), 115.8(d), 116.5(d), 117.5(d), 121.5(d), 121.6(d), 122.4*(d), 124.6(s), 127.9(s), 129.0(d), 129.2(d), 131.6(d), 132.6(d), 133.9(s), 139.4(s), 141.7(s), 143.6(s), 146.5(s), 148.4(s), 151.8(s), 152.6(s), 156.1(s). (*overlapped signals of 2 carbons). FT-IR $\upsilon^{KBr}cm^{-1}$: 3439, 2930, 2855, 1609, 1508, 1443, 1260, 1231, 1127. EI-MS *m/z* (rel. int.): 438 $[M]^+$(100), 301(60), 211(45). UV λ_{max} (EtOH) nm (log ε): 215 (4.62), 280 (4.03).

***Riccarin C triacetate* (24).** A mixture including riccardin C (**5**, 30.7 mg) was treated in the same manner as described above to yield **24** (35.4 mg) which was purified by CC on silica gel (*n*-hexane-EtOAc gradient). White powder, ^{1}H- and ^{13}C-NMR δ: see Tables 1 and 2. FT-IR $\upsilon^{KBr}cm^{-1}$: 3029, 2932, 2860, 1765, 1593, 1505, 1370, 1202, 1013, 756. EI-MS *m/z* (rel. int.): 550 $[M]^+$(39), 508(11), 466(100), 424(73), 211(67). UV λ_{max} (dioxane) nm (log ε): 236 (4.29).

***Acid hydrolysis of* 10.** A solution of **10** (4.7 mg) in 5% H_2SO_4 (3 ml) in EtOH (1 ml) was heated at 100° for 2 hr. The reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was evapd *in vacuo* to afford the aglycone (2.4 mg), identified as apigenin through comparison of its spectral data with those reported in ref.16. The aq. layer was neutralized with Amberlite IR-45 and evapd *in vacuo*. The existence of D-glucose was confirmed by using RI detection (Waters 410) and chiral detection (Shodex OR-1), respectively, on HPLC (Shodex RSpak DC-613, 80% MeCN, column temp.; 70°, flow rate; 0.8 ml min^{-1}) by comparison with the authentic sugar (10 mM of D-glucose). The sugar part gave 1 peak indicating a positive optical rotation at 15.0 min. (D-glucose, 14.9 min).

***Shikimic acid* (11).** Light yellow crystals, m.p.: 152.0-153.0°. FT-IR $\upsilon^{KBr}cm^{-1}$: 3500-2500, 1688, 1233, 1117, 1030. EI-MS *m/z* (rel. int.): 174 $[M]^+$(5), 156(26), 138(33), 127(12), 115(41), 110(29), 97 (100), 82(16), 69(40), 60(74). UV (MeOH) nm (log ε): 213 (3.90). $[\alpha]_D$: -153.5° (*c* 0.50, MeOH) [lit. 17, -157°].

Evaluation of activity. Compounds **2**, **3**, **5** and **6** were evaluated for their potential to inhibit enzyme activity[18] and their cytotoxic potential[19], as described previously.

Acknowledgements. We thank Dr. M. Mizutani (The Hattori Botanical Laboratory, Nichinan, Japan) for the identification of *B. pusilla* and Miss Y. Okamoto (TBU) for the measurement of mass spectra and Dr. K. Yoshikawa (TBU) for HPLC analysis. Thanks are due to Prof. S. Arihara and Dr. M. Toyota (TBU) for their helpful discussions. This work was partly supported by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan.

REFERENCES AND NOTES

1. Current address: Suntory Institute for Bioorganic Resarch, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618, Japan.
2. (a) Asakawa, Y. in *Progress in the Chemistry of Organic Natural Products* (Herz, W.; Kirby, G. W.; Moore, R. E.; Steglich, W.; Tamm, Ch. eds.), **1995**, Vol. 65, p. 1-562, Springer, Wien.
(b) Asakawa, Y. in *Bioactive Natural Products: Detection, Isolation, and Structural Determination*

(Colegate, S. M.; Molyneux, R. J. eds.), **1993**, p. 319-347, CRC Press, Florida.

3. (a) Hashimoto, T.; Yoshida, T.; Kan, Y.; Takaoka, S.; Tori, M.; Asakawa, Y. *Tetrahedron Letters*, **1994**, *35*, 909-910.
(b) Hashimoto, T.; Yoshida, T.; Tori, M.; Takaoka, S.; Asakawa, Y. **1993,** *XV International Botanical Congress. Yokohama, Japan. Abstract*, p. 4047.
(c) Hashimoto, T.; Yoshida, T.; Kanayama, S.; Takaoka, S.; Kan, Y.; Fukuyama, Y.; Tori, M.; Asakawa, Y. *35th Tennen Yuki Kagobutsu Toronkai. Kyoto, Japan. Symposium Papers.*, **1993**, p.353-360.
4. Mues, R. in *Bryophytes : Their Chemistry and Chemical Taxonomy. Proc. Phytochemical Society of Europe* (Zinsmeister H. D. and Mues R. eds.), **1990**, p.421-435, Clarendon Press, Oxford.
5. Kunz, S.; Becker, H. *Phytochemistry*, **1994**, *36*, 675-677.
6. Asakawa, Y.; Matuda, R. *Phytochemistry*, **1982**, *21*, 2143-2144.
7. Asakawa, Y.; Toyota, M; Bischler, H; Campbell, E. O.; Hattori, S. *J. Hattori Bot. Lab.*, **1984**, *57*, 383-389.
8. Gorham, J. *Phytochemistry*, **1977**, *16*, 249-253.
9. Valio, I. F. M.; Burdon, R. S.; Schwabe, W. W. *Nature*, **1969**, *223*, 1176-1178.
10. Adesanya, S. A.; Ogundana, S. K.; Roberts, M. F. *Phytochemistry*, **1989**, *28*, 773-774.
11. Redaelli, C.; Formentini, L.; Santaniello, E. *Phytochemistry* **1980**, *19*, 985-986.
12. Maass, W. S. G. *Phytochemistry*, **1975**, *14*, 2478-2489.
13. Arnone, A.; Cardillo, R.; Nasini, G. *Phytochemistry*, **1986**, *25*, 471-474.
14. Huneck, S.; Schreiber, K.; Sundholn, G. *Phytochemistry*, **1980**, *19*, 886-887.
15. (a) Ebizuka, Y.; Sankawa, U.; Shibata, S. *Phytochemistry*, **1970**, *9*, 2061-2063.
(b) Kim, Y. C.; Bolzani, V. S.; Baj, N.; Gunatilaka, A. A. L.; Kingston, D. G. I. *Planta Medica*, **1996**, *62*, 61-63.
16. Achari, B.; Chaudhuri, C.; Saha, C. R.; Dutta, P. K.; Pakrashi S. C. *Phytochemistry* **1990**, *29*, 3671-3673.
17. The Merk Index, Ninth Edition **1976**, p. 8224.
18. Pengsuparp, T.; Cai, L.; Constant, H.; Fong, H. H. S.; Lin, L. Z.; Kinghorn, A. D.; Pezzuto, J. M.; Cordell, G. A.; Ingolfsdottir, K.; Wagner, H.; Hughes, S. H. *J. Nat. Prod.*, **1995**, *58*, 1024-1031.
19. Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M.; Ruangrungsi, N. *J. Nat. Prod.*, **1993**, *56*, 30-38.

(*Received in Japan* 22 *August* 1996; *accepted* 24 *September* 1996)