Paeonianins A−E, New Dimeric and Monomeric Ellagitannins from the Fruits of *Paeonia lactiflora*

Takashi Tanaka, Masatake Fukumori, Tomoko Ochi, and Isao Kouno*

Department of Molecular Medicinal Sciences, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

Received December 31, 2002

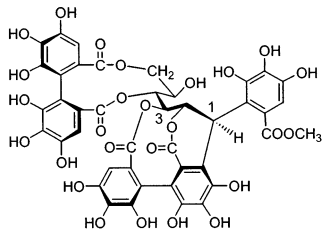
Four new dimeric ellagitannins, paeonianins A−D (**2**−**5**), were isolated from the fruits of *Paeonia lactiflora*, together with a new ellagitannin monomer, paeonianin E (**1**). Their structures were determined by spectroscopic methods. Paeonianins A−D (**2**−**5**) are positional isomers formed by condensation of pentagalloyl-*β*-D-glucose (**8**) with 5-desgalloylstachyurin (**6**) or casuariin (**7**). Paeonianin E is a *C*-glycosidic ellagitannin having a gallic acid methyl ester moiety at the glucose C-1 position. This is the first report of the isolation of dimeric ellagitannins from a plant in the family Paeoniaceae.

Paeoniae lactiflora Pall. (Paeoniaceae) is an important ornamental and medicinal plant in both Japan and mainland China. The roots of *Paeonia* species are widely used in traditional medicine, and there are many reports of the various chemical constituents, mainly monoterpenoid glycosides[1−4] and tannins.[1,5] In contrast, the constituents of the fruits of plants in this genus have not been studied in detail because they are not used medicinally. In a preliminary examination, the presence of polar ellagitannins was suggested in the fruits of *P. lactiflora*, together with large amounts of polygalloylglucoses and the monoterpene glycoside paeoniflorin. As a part of our study on polyphenols in foods and medicinal herbs,[6−9] we separated the constituents of the fruits and have succeeded in isolating and characterizing five new ellagitannins named paeonianins A−E (**1**−**5**). This paper describes the isolation and structure elucidation of these compounds.

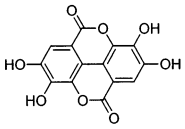
Results and Discussion

The fresh fruits of *P. lactiflora* were crushed, extracted with methanol, and partitioned successively with *tert*-butyl methyl ether and EtOAc. The *tert*-butyl methyl ether extract contained mainly gallic acid and polygalloylglucoses. The EtOAc extract and the water layer were separately subjected to sequential column chromatography over Sephadex LH-20, MCI gel CHP20P, TSK gel Toyopearl HW40F, Funacel microcrystalline cellulose, and Chromatorex ODS to yield paeonianins A−E (**1**−**5**), along with 17 known compounds. The known compounds were identified as two quinic acid gallates (4-*O*-galloyl and 5-*O*-galloyl quinic acids),[10,11] six galloyl glucoses (1-*O*-,[12] 1,2,3-tri-*O*-,[13] 1,2,6-tri-*O*-,[9] 1,3,6-tri-*O*-,[14] 1,2,3,6-tetra-*O*-,[5] and 1,2,3,4,6-penta-*O*-[5] (**8**) galloyl-*β*-D-glucoses), and nine ellagitannins [2,3-(*S*)-hexahydroxydiphenoyl (HHDP)-D-glucose,[13] sanguiin H-5,[15] strictinin,[9] 5-desgalloyl stachyurin (**6**),[13] casuariin (**7**),[13] pedunculagin,[13] 1-desgalloyl eugeniin,[13] eugeniin,[13] and 1(*β*)-*O*-galloyl pedunculagin[15]] by comparison of their spectral data with those of authenticated samples.

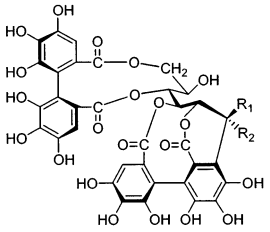
Paeonianin E (**1**) was obtained as a white amorphous powder and characterized as an ellagitannin by standard color reactions with a nitrite−acetic acid reagent (reddish brown) and a ferric chloride reagent (dark blue).[16] The ^{1}H NMR spectrum (Table 1) showed seven proton signals due to five aliphatic methines and a methylene, the coupling



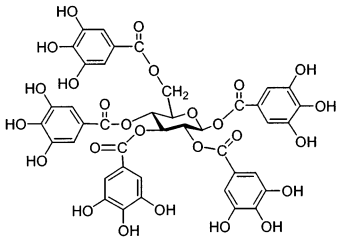
1



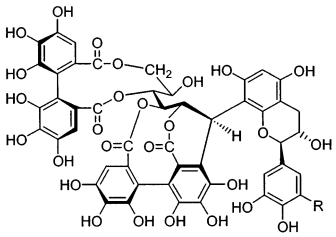
1a



R$_1$ R$_2$
6 : OH H
7 : H OH



8



R
9 : H
10 : OH

constants of which were closely related to those of 5-desgalloyl stachyurin (**6**) ($J_{1,2}$, $J_{2,3}$, $J_{3,4}$ < 2 Hz, $J_{4,5}$ = 7.5 Hz, $J_{5,6}$ = 3.0 Hz and < 1 Hz), suggesting the presence of a *C*-glycosidic glucose moiety. In addition, the chemical shift of the signals indicated esterification at C-2, C-3, C-4, and C-6 of the glucose moiety. On the other hand, the chemical shifts of the glucose carbon signals in the ^{13}C NMR spectrum (Table 2) resembled those of stenophyllanin C (**9**)[17,18] and strobilanin (**10**)[17,19] rather than those of **6** [*δ* 65.36 (C-1), 80.78 (C-2), 73.07 (C-3), 76.26 (C-4), 68.84 (C-5), 67.97 (C-6)], indicating that the glucose C-1 (*δ* 41.3) of **1** was connected to two aromatic rings. The presence of HHDP esters in the molecule of **1** was suggested by acid

* To whom correspondence should be addressed. Tel: +81-95-819-2432. Fax: +81-95-819-2432. E-mail: ikouno@net.nagasaki-u.ac.jp.

10.1021/np020608g CCC: $25.00
Published on Web 05/20/2003

Table 1. ^{1}H NMR Spectral Data for Compounds **1**−**6** and **8** (measured at 500 MHz in acetone-d_6+D_2O)

	1[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[b]	**8**[b]
glc-1[c]							
1	5.10 (br s)	4.53 (br s)	4.42 (br s)	4.79 (br s)	5.25 (br s)	4.90 (d, 1.8)	
2	4.92 (br s)	5.02 (dd, 1.2, 1.9)	4.93 (dd, 1.1, 1.8)	5.00 (dd, 1.2, 1.9)	4.93 (br s)	4.95 (t-like, 2.0)	
3	5.37 (br s)	5.24 (t, 1.9)	5.20 (t, 1.8)	5.49 (br s)	5.25 (br s)	4.91 (t-like, 3.0)	
4	5.53 (br d, 7.5)	5.82 (dd, 1.9, 7.3)	5.80 (dd, 1.8, 7.3)	5.65 (dd, 2.2, 7.7)	5.38 (dd, 2.7, 8.2)	5.19 (dd, 3.2, 8.4)	
5	4.16 (dd, 3.0, 7.5)	4.28 (br ddd, 3.0, 7.3, 7.5)	4.27 (br d, 7.3)	4.27 (br d, 7.7)	4.14 (dd, 3.2, 8.2)	4.11 (dd, 3.0, 8.5)	
6	3.87 (d, 12.5)	4.93 (dd, 3.0, 12.6)	4.90 (dd, 3.0, 12.3)	4.85 (dd, 3.0, 12.4)	4.71 (dd, 3.2, 12.1)	4.69 (dd, 3.0, 12.0)	
	4.80 (dd, 3.0, 12.5)	3.97 (br d, 12.6)	3.98 (br d, 12.3)	3.97 (br d, 12.4)	3.82 (br d, 12.1)	3.84 (br d, 12.0)	
OH-5		5.64 (d, 7.5)					
BP-H-3	6.52 (s)	6.52 (s)	6.52 (s)	6.54 (s)	6.46 (s)	6.42 (s)	
HHDP-H-3	6.88 (s)	6.91 (s)	6.90 (s)	6.92 (s)	6.73 (s)	6.68 (s)	
HHDP-H-3′	6.56 (s)	6.57 (s)	6.67 (s)	6.72 (s)	6.51 (s)	6.51 (s)	
GM-H-6	7.13 (br s)						
CH3	3.81 (s)						
glc-2							
1		6.32 (d, 8.2)	6.37 (d, 8.2)	6.37 (d, 8.5)	6.38 (d, 8.2)		6.33 (d, 8.4)
2		5.74 (dd, 8.2, 9.6)	5.59 (dd, 8.2, 9.8)	5.69 (dd, 8.5, 9.8)	5.64 (dd, 8.2, 9.8)		5.61 (dd, 8.4, 9.8)
3		5.96 (t, 9.6)	6.14 (t, 9.8)	6.22 (t, 9.8)	6.03 (t, 9.8)		6.01 (t, 9.8)
4		5.65 (t, 9.6)	5.71 (t, 9.8)	5.73 (t, 9.8)	5.65 (t, 9.8)		5.66 (t, 9.8)
5		4.56 (ddd, 2.1, 4.5, 9.6)	4.63 (ddd, 1.8, 4.8, 9.8)	4.57 (ddd, 2.1, 6.6, 9.8)	4.55 (m)		4.55 (m)
6		4.53 (dd, 2.1, 12.6)	4.56 (dd, 1.8, 12.6)	4.49 (br d, 12.4)	4.55 (m)		4.55 (m)
		4.46 (dd, 4.5, 12.6)	4.39 (dd, 4.8, 12.6)	4.42 (dd, 6.6, 12.4)	4.32 (br d, 10.5)		4.40 (dd, 4.6, 12.6)
galloyl (C-1)		6.94 (2H, s)	7.12 (2H, s)	7.13 (2H, s)	7.13 (2H, s)		7.18 (2H, s)[d]
galloyl (C-2)		6.85 (1H, s)	7.15 (2H, s)	7.04 (2H, s)	7.05 (2H, s)[d]		7.12 (2H, s)[d]
galloyl (C-3)		7.03 (2H, s)	6.84 (1H, s)	6.93 (2H, s)	7.04 (2H, s)		7.06 (2H, s)[d]
galloyl (C-4)		7.04 (2H, s)	6.91 (2H, s)	6.54 (1H, s)	6.98 (2H, s)[d]		7.02 (2H, s)[d]
galloyl (C-6)		7.18 (2H, s)	7.21 (2H, s)	7.17 (2H, s)	7.35 (1H, s)		6.97 (2H, s)[d]

[a] Measured at 500 MHz in acetone-d_6. [b] Measured in 300 MHz in acetone-d_6. [c] For numbering, see Figures 1 and 2. [d] Assignments may be interchanged in each column.

Table 2. ^{13}C NMR Spectral Data for Sugar Carbons of **1**−**5**, **8**, and **9** (measured at 125 MHz in acetone-d_6+D_2O)

	1	**2**	**3**	**4**	**5**	**8**	**9**
glc-1							
1	41.25	41.83	41.74	41.50	40.90		38.1
2	82.56	81.82	81.80	81.71	81.92		80.9
3	76.06	76.56	76.70	76.15	76.45		76.1
4	76.39	78.87	78.12	77.69	76.45		76.1
5	68.58	69.30	68.99	68.74	68.69		68.8
6	67.84	67.36	67.20	67.64	67.94		67.7
glc-2							
1		93.52	93.41	93.26	93.47	93.3	
2		71.53	71.83	71.95	71.86	71.7	
3		73.15	72.94	73.31	73.40	73.3	
4		69.45	69.57	69.58	69.44	69.2	
5		74.00	73.67	73.90	73.80	73.9	
6		62.59	62.95	63.22	62.92	62.8	

hydrolysis liberating ellagic acid (**1a**). Comparison of the aromatic carbon signals of **1** with those of **9** and **10** revealed the presence of two HHDP groups, one of which (BP in Figure 1) was connected to glucose C-1 through a C−C bond. The HMBC correlations illustrated in Figure 1 were used to assign unequivocally the positions where these HHDP ester groups were attached. The remaining eight carbon [δ 121.0 (C-1), 121.2 (C-2), 146.8 (C-3), 137.4 (C-4), 143.9 (C-5), 111.2 (C-6), 168.8 (C-7), 52.2 (CH_3)] and two proton [δ 7.13 (1H, br s)[20] and 3.78 (3H, s)] signals, as well as their long-range ^{1}H−^{13}C correlations (Figure 1), indicated the occurrence of a gallic acid methyl ester moiety at glucose C-1 (GM in Figure 1). The [M − H]$^-$ peak at *m*/*z* 949 in the FABMS (negative-ion mode) was consistent

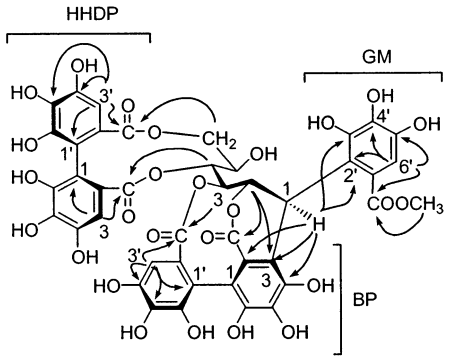


Figure 1. Important HMBC correlations observed for **1**.

with this structural assignment. The atropisomerism of the two biphenyl bonds was determined to be *S* in both cases, because the CD spectrum of **1** showed a negative Cotton effect at 261 nm and a positive Cotton effect at 235 nm.[21] On the basis of these spectroscopic results, the structure of paeonianin E was determined to be as represented by formula **1**.

Paeonianins A−D (**2**−**5**) were also characterized as ellagitannins by their color reactions with nitrite−acetic acid reagent and ferric chloride reagent. The negative-ion FABMS of **2**−**5** exhibited their [M − H]$^-$ peaks at *m*/*z* 1705, indicating that these tannins are ellagitannin dimers with the same molecular mass. The ^{1}H and ^{13}C NMR spectra of **2**−**5** also resembled each other. Accordingly, in the ^{1}H NMR spectrum of each compound, four two-proton singlets due to galloyl groups and four one-proton singlets appeared in

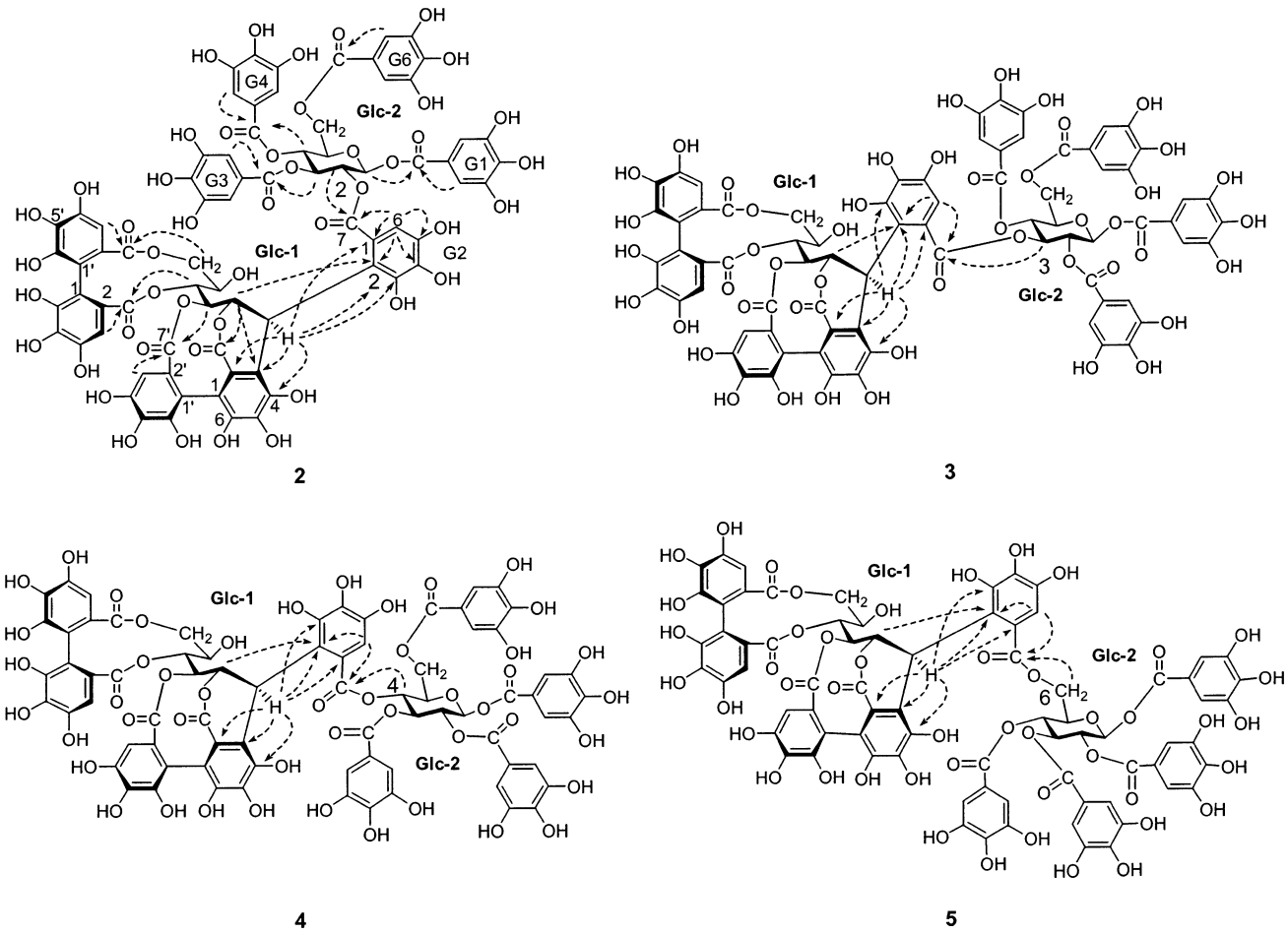


Figure 2. Structures of **2**−**5** and the key HMBC correlations.

the aromatic region, and 14 signals arising from two glucose moieties were observed in the aliphatic region (Table 1). Chemical shifts and large coupling constants ($J_{1,2}$ = 8 Hz, $J_{2,3}$, $J_{3,4}$, $J_{4,5}$ = 9−10 Hz) of the seven signals due to one of the glucoses were similar to those of 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucopyranose (**8**), suggesting the presence of a fully acylated $^4C_1$-*β*-glucopyranose in each molecule (Glc-2 in Table 1 and Figure 2). On the other hand, chemical shifts and coupling constants of the signals arising from the other glucose moiety were related to the linear glucose of **1** (Glc-1 in Table 1 and Figure 2). The presence of these two types of glucose units in the molecules of **2**−**5** was supported by ^{13}C NMR spectral comparisons of their sugar carbon signals with those of **1** and **8** (Table 2).

Compounds **2**−**5** all exhibited aromatic and carboxyl carbon NMR signals due to galloyl and HHDP groups. Comparison of the signals with those of **1** revealed that one of the HHDP groups was linked to a sugar residue through a C−C bond. In addition, signals due to one of the galloyl groups coincided with those of the C-2 substituted gallic acid methyl ester moiety of **1** (GM in Figure 1). The appearance of these carbon signals suggested the presence of a paeonianin E (**1**) unit in each molecule, which was confirmed by the observation of $^1H-^{13}C$ long-range coupling in each HMBC spectrum (Figure 2). In the case of paeonianin A (**2**), the location of the two HHDP ester groups was evident from the correlations between the sugar protons and the carbonyl carbons, which were similar to those observed for **1**. The H-1 signal of the linear glucose (Glc-1) correlated with six aromatic carbons, three of which were attributable to C-2, C-3, and C-4 of the *C*-glycosidically linked HHDP group. The remaining three carbons were assignable to C-1, C-2, and C-3 of the C-2 substituted gallic acid moiety by comparison of the chemical shifts with those of **1** and their correlations with the aromatic singlet at *δ* 6.85 (G2 H-6 in Figure 2). The C-2 signal of the gallic acid moiety also correlated with H-2 of the linear glucose (Glc-1) through ^3J coupling. These HMBC correlations unequivocally indicated the presence of a paeonianin E unit in the molecule of **2**. The aromatic proton of the *C*-glycosidically linked galloyl group (*δ* 6.85, H-6 of G2 aromatic ring) was correlated with a carboxyl carbon at *δ* 167.37, which was in turn correlated with H-2 of the $^4C_1$-*β*-glucopyranose moiety (Glc-2 in Figure 2). This observation indicated that the paeonianin E unit was attached to the C-2 position of the $^4C_1$-glucopyranose moiety in **2**. The locations of the remaining four galloyl groups on glucopyranose were deduced from the ^3J correlations from the galloyl protons and glucose methine protons to galloyl carboxyls. Although no correlation was observed for H-6 of $^4C_1$-glucopyranose, esterification of this position was apparent from the chemical shifts (Table 1). In addition, partial hydrolysis of **1** in hot water afforded 1,3,4,6-tetra-*O*-galloyl-*β*-D-glucopyranose. [22] Therefore, the structure of paeonianin A was determined to be as represented by formula **2**. Based on similar spectroscopic observations, the locations of the paeonianin E (**1**) units in paeonianins B−D (**3**−**5**) were determined to be at C-3, C-4, and C-6, respectively, of the $^4C_1$-glucopyranose moieties, and their structures are represented by formulas **3**−**5** in Figure 2.

Paeonianins A−D are probably generated by condensation of 5-desgalloyl stachurin (**6**) or casuariin (**7**) with pentagalloyl-*β*-D-glucose (**8**). Although ellagitannins having

structures related to paeonianins A−D have been isolated from many plant sources,[23] to the best of our knowledge, this is the first report of the isolation of dimeric ellagitannins from a plant in the family Paeoniaceae. The possibility of paeonianin E (**1**) being an artifact generated from **2**−**5** during extraction with methanol was considered. However, the heating of **2**−**5** with methanol containing acetate buffer[24] yielded complex mixtures, and **1** was not detected among the products.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. CD spectra were measured with a JASCO J-720W apparatus. ^{1}H and ^{13}C NMR spectra were recorded in acetone-d_6 with Varian Unity plus 500 and Varian Gemini 300 spectrometers operating at 500 and 300 MHz for ^{1}H, and 125 and 75 for ^{13}C, respectively. Coupling constants are expressed in Hz, and chemical shifts are given on a δ (ppm) scale with tetramethylsilane as the internal standard. MS were recorded on a JEOL JMS DX-303 spectrometer, and glycerol was used as a matrix for FABMS measurement.

Column chromatography was performed with MCI-gel CHP 20P (75−150 μm, Mitsubishi Chemical Co.), Sephadex LH-20 (25−100 μm, Pharmacia Fine Chemical Co. Ltd.), TSK gel Toyopearl HW-40F (TOSOH), Funacel microcrystalline cellulose (Funakoshi), and Chromatorex ODS (100−200 mesh, Fuji Silysia Chemicasl Ltd.). TLC was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merck) or on precoated cellulose plates (Merck), and spots were detected by ultraviolet (UV) illumination and by spraying with 2% ethanolic $FeCl_3$ and 10% sulfuric acid reagent. HPLC performed on a Cosmosil 5C_{18}-AR II (Nacalai Tesque Inc.) column (250 × 4.6 mm i.d.) with gradient elution from 4% to 30% (39 min) and 30% to 75% (15 min) of CH_3CN in 50 mM H_3PO_4 (flow rate, 0.8 mL/min; detection, JASCO photodiode array detector MD-910).

Plant Material. The fruits of *P. lactiflora* were collected from the Medicinal Plants Garden of Nagasaki University, in June 1999, where a voucher specimen was deposited (NAP0406−99/5).

Extraction and Isolation. Fresh fruits (2.65 kg) were crushed and extracted three times with MeOH to give an extract (339 g). The dried MeOH extract was suspended in water and partitioned successively with *tert*-butyl methyl ether[25] and EtOAc. The *tert*-butyl methyl ether extract (140.8 g) was repeatedly separated by Sephadex LH-20 column chromatography with EtOH containing increasing amounts of water and then water−acetone (1:1, v/v) to give gallic acid[26] (29.9 g) and a mixture of polygalloylglucoses (63.7 g). The EtOAc extract (31.4 g) was chromatographed over Sephadex LH-20 with water containing increasing amounts of MeOH and then aqueous acetone (1:1, v/v) to afford three fractions: fractions A1 (8.5 g), A2 (7.63 g), and A3 (18.9 g). Fraction A1 was mainly composed of paeoniflorin[1] and sugars. Fraction A2 was separated by a combination of column chromatography over MCI gel CHP20P (H_2O−MeOH), Funacel (2% acetic acid), Chromatorex ODS (H_2O−MeOH), and Sephadex LH-20 (H_2O−MeOH) to yield 1,2,3-tri-*O*-galloyl-β-D-glucose[13] (158 mg), 1,2,6-tri-*O*-galloyl-β-D-glucose[9] (153 mg), 1,3,6-tri-*O*-galloyl-β-D-glucose[14] (37 mg), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose[5] (45 mg), eugeniin[13] (787 mg), 1-desgalloyleugeniin[13] (173 mg), 1(β)-*O*-galloylpedunculagin[15] (631 mg), and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose[5] (**8**) (664 mg). Fraction A3 was mainly composed of eugeniin and galloylglucoses, and separation of a portion (1.1 g) of the fraction over Funacel (2% acetic acid) and MCI gel CHP20P (H_2O−MeOH) gave eugeniin (141 mg) and **8** (320 mg).

The aqueous layer was concentrated by evaporation under reduced pressure, and the residue (158.8 g) was applied to a column of MCI gel CHP20P with water containing increasing proportions of MeOH to give four further fractions: W1 (129.3 g), W2 (17.5 g), and W3 (25.2 g). Fraction W1 contained sugars as major constituents; chromatography of this faction over Sephadex LH-20 (H_2O−20% MeOH) and MCI gel CHP20P (H_2O−25% MeOH) yielded four phenolic substances, which were identified as 3-*O*-galloyl quinic acid[10,11] (290 mg), 4-*O*-galloyl quinic acid (50.6 mg), 1-*O*-galloyl-β-D-glucose[12] (149 mg), and 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose[13] (266 mg). Fraction W2 was further separated by a combination of chromatography on Sephadex LH-20 (H_2O−MeOH), MCI gel CHP20P (H_2O−MeOH), TSK gel Toyopearl HW40F (H_2O−MeOH), Funacel (2% acetic acid), and Chromatorex ODS (H_2O−MeOH), to yield pedunculagin[13] (5.8 g), 5-desgalloylstachyurin[13] (**6**) (406 mg), casuariin[13] (**7**) (268 mg), sanguiin H-5[15] (159 mg), and paeonianin E (**1**) (200 mg). Fraction W3 was subjected to Sephadex LH-20 column chromatography, and elution with H_2O afforded a crude crop of paeoniflorin (10.9 g). Further elution of the column with aqueous MeOH (40% to 100%) yielded three fractions: fractions W1-1 (1.5 g), W1-2 (3.7 g), W1-3 (3.0 g). On chromatographic separation similar to that described for fraction W2, these fractions yielded strictinin[9] (202 mg) from W1-1, 1-desgalloyleugeniin (342 mg) from W1-2, and eugeniin (749 mg), 1(β)-*O*-galloylpedunculagin (605 mg), **8** (167 mg), and paeonianins A (**2**) (29 mg), B (**3**) (223 mg), C (**4**) (380 mg), and D (**5**) (29 mg) from W1-3.

Paeonianiin E (1): white amorphous powder; $[\alpha]_D$ +166.9° (*c* 0.7, MeOH); CD (EtOH) $\Delta\epsilon$ (nm) −5.5 (313), +13.5 (285), −27.9 (261), +87.0 (235); ^{1}H NMR data, Table 1; ^{13}C NMR (acetone-d_6) δ 170.40 (BP-7′), 169.45 (HHDP-7′), 168.78 (GM-7), 168.32 (HHDP-7), 168.17 (BP-7), 146.82 (GM-3), 145.60 (BP-4′), 145.07, 145.01 (HHDP-4,4′), 144.08, 144.03, 143.94, 143.90 (HHDP-6,6′, GM-5, BP-6), 142.88 (BP-4), 142.71 (BP-6′), 137.53 (BP-5), 137.39 (GM-4), 136.17 (HHDP-5), 135.84 (HHDP-5′), 135.06 (BP-5′), 128.21, 127.24, 125.74 (BP-2′, HHDP-2,2′), 123.56 (BP-2), 122.90 (BP-3), 121.18 (GM-2), 121.04 (GM-1), 116.93 (BP-1′), 115.96 (BP-1), 115.41(2C) (HHDP-1,1′), 111.22 (GM-6), 108.79 (HHDP-3), 107.25 (HHDP-3′), 105.89 (BP-3′); see Table 2 for sugar ^{13}C NMR data; FABMS (negative-ion mode) *m/z* 949 $(M - H)^-$; *anal.* C 47.65%, H 4.00%, calcd for $C_{42}H_{30}O_{26}\cdot 6H_2O$ C 47.24%, H 4.05%.

Acid Hydrolysis of Compound 1. Compound **1** (10 mg) was dissolved in 5 mL of 0.5 M HCl and heated at 90 °C for 6 h. HPLC analysis of the reaction mixture with photodiode array detection showed a peak corresponding to ellagic acid (t_R 30.6 min, UV λ_{max} 251, 367 nm). After cooling, the resulting yellow needles (2.1 mg) were collected by filtration and identified as ellagic acid by IR spectral comparison with an authentic sample: mp > 300 °C; IR ν_{max} (KBr) 3400, 1720, 1690, 1610 cm^{-1}.

Paeonianin A (2): white amorphous powder; $[\alpha]_D$ +219.7° (*c* 0.7, MeOH); ^{1}H NMR data, Table 1; ^{13}C NMR (acetone-d_6) δ 172.19 (HHDP-7), 170.32 (BP-7′), 169.01 (HHDP-7′), 167.37 (G2-7), 167.18 (BP-7), 166.36 (G6-7), 165.90 (G3-7), 165.59 (G4-7), 165.19 (G1-7), 147.13 (G2-3), 146.01 (2C) (G6-3,5), 145.93 (2C) (G4-3,5), 145.71 (2C) (G3-3,5), 145.65 (2C) (G1-3,5), 145.57 (BP-4′), 145.40 (HHDP-4), 145.29 (HHDP-4′), 144.14 (2C), 144.07, 143.98, 143.12 (G2-5, BP-6,6′, HHDP-4,4′), 142.88 (BP-4), 139.93 (G1-4), 139.32, 139.18 (G3-4, G4-4), 139.03 (G6-4), 137.96 (G2-4), 137.17 (BP-5), 136.92 (2C) (HHDP-5,5′), 135.06 (BP-5′), 128.28, 127.05 (HHDP-2,2′), 124.97 (BP-2′), 124.00 (BP-2), 121.81 (BP-3), 121.37, 121.26 (G2−1,2) 121.18 (G6-1), 120.83 (G3-1), 120.48 (G4-1), 119.32 (G1-1), 116.71 (BP-1′), 116.00 (BP-1), 115.27 (HHDP-1′), 115.09 (HHDP-1), 110.32 (3C) (G2-6, G3-2,6), 110.23 (2C) (G4-2,6), 110.03 (2C) (G6-2,6), 109.88 (2C) (G1-2,6), 109.64 (HHDP-3), 108.25 (HHDP-3′), 105.99 (BP-3′); see Table 2 for sugar ^{13}C NMR data; FABMS (negative-ion mode) *m/z* 1705 $(M - H)^-$; *anal.* C 47.42, H 3.96, calcd for $C_{75}H_{54}O_{47}\cdot 10H_2O$ C 47.73%, H 3.95%.

Partial Hydrolysis of Compound 2. A solution of **1** (25 mg) in 5 mL of H_2O was heated at 85 °C for 12 h. The mixture was subjected to Sephadex LH-20 column chromatography with H_2O containing increasing proportions of MeOH to give gallic acid (3.1 mg) and 1,3,4,6-tetra-*O*-galloyl-β-D-glucopyranose (2.7 mg): white amorphous powder; $[\alpha]_D$ +4.5° (*c* 0.1, EtOH); ^{1}H NMR (acetone-d_6) δ 7.206, 7.155, 7.071, 7.044 (s, each 2H, galloyl H-2,6), 6.132 (d, 1H, J = 8.2 Hz, glc H-1), 5.686 (t, 1H, J = 9.5 Hz, glc H-3), 5.488 (t, 1H, J = 9.5 Hz, glc

H-4), 5.313 (d, 1H, $J = 5.2$ Hz, glc C-2-OH), 5.495 (d, 1H, $J = 10.9$ Hz, glc H-6), 4.28−4.39 (m, 2H, glc H-5, H-6), 4.049 (ddd, 1H, $J = 5.2, 8.2, 9.5$ Hz, glc H-2). The ^{1}H NMR data and the $[\alpha]_D$ value were identical with those reported in the literature.[22]

Paeonianin B (3): white amorphous powder; $[\alpha]_D$ +158.40° (*c* 0.5, MeOH); ^{1}H NMR data, Table 1; ^{13}C NMR (acetone-d_6) δ 171.28 (HHDP-7), 170.69 (BP-3′), 169.2 (HHDP-7′), 168.09 (BP-7), 167.27 (G3-7), 167.18 (G4-7), 166.55 (G6-7), 165.90 (G2-7), 165.11 (G1-7), 146.84 (G3-3), 146.00 (2C) (G1-3,5), 145.87 (2C) (G6-3,5), 145.52 (2C), 145.44 (3C) (G2-3,5, G4-3,5, BP-4′), 145.20 (HHDP-4′), 144.12 (2C), 143.80, 143.74 (2C) (G3-5, BP-6, HHDP-4,6,6′), 142.79 (2C) (BP-4,6′), 139.69 (G1-4), 139.61 (G4-4), 139.38 (G2-4), 138.89 (G6-4), 137.56 (G3-4), 136.98 (BP-5), 136.68 (HHDP-5′), 136.60 (HHDP-5), 135.04 (BP-5′), 127.94, 126.96 (HHDP-2,2′), 124.90 (BP-2′), 123.56 (BP-2), 121.66 (BP-3), 121.18 (G6-1), 120.82, 120.80 (G3-1,2), 120.27 (G2-1), 119.88 (G4-1), 119.56 (G1-1), 116.69 (BP-1′), 115.78 (BP-1), 115.21 (HHDP-1′), 114.92 (HHDP-1), 110.46 (2C) (G2-2, 6), 110.19 (3C) (G1-2,6, G3-6), 109.96 (2C), 109.90 (2C) (G4-2,6, G6-2,6), 109.41 (HHDP-4), 107.87 (HHDP-3′), 105.92 (BP-3′); see Table 2 for sugar ^{13}C NMR data; FABMS (negative-ion mode) *m*/*z* 1705 $(M - H)^-$; *anal*. C 47.13%, H 3.95%, calcd for $C_{75}H_{54}O_{47}\cdot 11H_2O$ C 47.28%, H 4.02%.

Paeonianin C (4): white amorphous powder; $[\alpha]_D$ +115.0° (*c* 0.7, MeOH); ^{1}H NMR data, Table 1; ^{13}C NMR (acetone-d_6) δ 170.50 (BP-7′), 170.39 (HHDP-7), 169.41 (HHDP-7′), 168.08 (BP-7), 167.99 (G4-7), 167.30 (G3-7), 166.88 (G6-7), 166.08 (G2-7), 165.31 (G1-7), 146.96 (G4-3), 146.02 (2C) (G1-3,5), 145.85 (4C) (G2-3,5, G6-3,5), 145.63 (BP-4′), 145.49 (G3-3,5), 145.23, 145.20 (HHDP-4,4′), 144.21, 144.20, 144.14, 144.11 (G4-5, BP-6, HHDP-6,6′), 142.99 (BP-4), 142.84 (BP-6′), 139.82 (G1-4), 139.51 (G3-4), 139.33 (G2-4), 139.08 (G6-4), 137.71 (G4-4), 137.46 (BP-5), 136.66 (HHDP-5), 136.40 (HHDP-5′), 135.14 (BP-5′), 128.00, 127.19 (HHDP-2,2′), 124.97 (BP-2′), 123.96 (BP-2), 121.90 (BP-3), 121.51 (G4-1), 120.98 (G6-1), 120.48 (G4-2), 120.06, 120.03 (G2-1, G3-1), 119.49 (G1-1), 116.83 (BP-1′), 115.92 (BP-1), 115.53 (HHDP-1), 115.29 (HHDP-1′), 110.56 (G4-6), 110.21 (2C) (G1-2,6), 110.03 (4C) (G2-2,6, G6-2,6), 109.97 (G3-2,6), 109.26 (HHDP-3), 107.83 (HHDP-3′), 105.96 (BP-3′); see Table 2 for sugar ^{13}C NMR data; FABMS (negative-ion mode) *m*/*z* 1705 $(M - H)^-$; *anal*. C 18.15%, H 3.91%, calcd for $C_{75}H_{54}O_{47}\cdot 9H_2O$ C 48.19%, H 3.88%.

Paeonianin D (5): white amorphous powder; $[\alpha]_D$ +79.0° (*c* 0.7, MeOH); ^{1}H NMR data, Table 1; ^{13}C NMR (acetone-d_6) δ 170.58 (BP-7′), 169.24 (HHDP-7′), 168.23 (HHDP-7), 167.62 (G6-7), 166.90 (BP-7), 166.02, 165.98 (G2-7, G3-7), 165.82 (G4-7), 165.45 (G1-7), 147.02 (G6-3), 145.98 (2C) (G1-3,5), 145.82 (2C), 145.79 (2C), 145.73 (2C) (G2-3,5, G3-3,5, G4-3,5), 145.45 (BP-4′), 144.98 (HHDP-4), 144.87 (HHDP-4′), 144.25 (HHDP-6), 144.08, 144.02 (HHDP-6′, BP-6), 143.75 (G6-5), 143.06 (BP-6′), 142.73 (BP-4), 139.75 (G1-4), 139.39, 139.03 (G2-4, G3-4), 139.18 (G4-4), 137.63 (BP-5), 137.49 (G6-4), 136.12 (HHDP-5), 135.68 (HHDP-5′), 134.90 (BP-5′), 128.43, 127.53 (HHDP-2,2′), 126.00 (BP-2′), 124.26 (BP-2), 122.84 (BP-3), 121.61 (G6-2), 120.79 (G6-1), 120.51, 120.40, 120.30 (G2-1, G3-1, G4-1), 119.75 (G1-1), 116.96 (BP-1′), 115.89 (BP-1), 115.44 (2C) (HHDP-1,1′), 111.75 (G6-6), 110.42 (2C), 110.37 2C), 110.07 (4C) (G1-2,6, G2-2,6, G3-2,6, G4-2,6), 108.44 (HHDP-3), 107.26 (HHDP-3′), 105.68 (BP-3′); see Table 2 for sugar ^{13}C NMR data; FABMS (negative-ion mode) *m*/*z* 1705 $(M - H)^-$; *anal*. C 48.34%, H 3.94%, calcd for $C_{75}H_{54}O_{47}\cdot 8H_2O$ C 48.66%, H 3.81%.

Acknowledgment. The authors are grateful to Mr. K. Inada and Mr. N. Yamaguchi (Nagasaki University) for NMR and MS measurements.

References and Notes

(1) Tanaka, T.; Kataoka, M.; Tsuboi, N.; Kouno, I. *Chem. Pharm. Bull.* **2000**, *48*, 201−207.
(2) Kostova, I. N.; Simeonov, M. F.; Todorova, D. I. *Phytochemistry* **1998**, *47*, 1303−1307.
(3) Murakami, N.; Saka, M.; Shimada, H.; Matsuda, H.; Yamahara, J.; Yoshikawa, M. *Chem. Pharm. Bull.* **1996**, *44*, 1279−1281.
(4) Lin, H.-C.; Ding, H.-Y.; Shung, T.; Wu, P.-L. *Phytochemistry* **1996**, *41*, 237−242.
(5) Nishizawa, M.; Yamagishi, T.; Nonaka, G.; Nishioka, I.; Nagasawa, T.; Oura, H. *Chem. Pharm. Bull.* **1983**, *31*, 2593−2600.
(6) Ling, S.-K.; Komorita, A.; Tanaka, T.; Fujioka, T.; Mihashi, K.; Kouno, I. *J. Nat. Prod.* **2002**, *65*, 656−660.
(7) Ling, S.-K.; Komorita, A.; Tanaka, T.; Fujioka, T.; Mihashi, K.; Kouno, I. *J. Nat. Prod.* **2002**, *65*, 656−660.
(8) Zhang, Y.-J.; Abe, T.; Tanaka, T.; Yang, C.-R.; Kouno, I. *J. Nat. Prod.* **2001**, *64*, 1527−1532.
(9) Jiang, Z.-H.; Hirose, Y.; Iwata, H.; Sakamoto, S.; Tanaka, T.; Kouno, I. *Chem. Pharm. Bull.* **2001**, *49*, 887−892.
(10) Numbering of the quinic acid ring followed the IUPAC nomenclature recommendations. *Biochem. J.* **1976**, *153*, 23−31.
(11) Nishimura, H.; Nonaka, G.; Nishioka, I. *Phytochemistry* **1984**, *23*, 2621−2623.
(12) Tanaka, T.; Nonaka, G.; Nishioika, I.; Miyahara, K.; Kawasaki, T. *J. Chem. Soc., Perkin Trans. 1* **1986**, 369−376.
(13) Lee, S.-H.; Tanaka, T.; Nonaka, G.; Nishioka, I. *Phytochemistry* **1990**, *29*, 3621−3625.
(14) Schmidt, O. T.; Schulz, J.; Fiesser, H. *Justus Liebigs Ann. Chem.* **1967**, *706*, 187−197.
(15) Tanaka, T.; Nonaka, G.; Nishioka, I. *J. Chem. Res. (S)* **1985**, 176−177; *(M)* **1985**, 2001−2029.
(16) Bate-Smith, E. C. *Phytochemistry* **1972**, *11*, 1153−1156.
(17) The ^{1}H NMR spectra of **9** and **10** measured at room temperature showed broadened signals due to dynamic rotational isomerism; therefore, the spectra of these compounds could not be compared with that of **1**. On the other hand, the ^{13}C NMR spectra of **9** and **10** measured at room temperature exhibited sharp signals. See ref 19.
(18) Nonaka, G.; Nishimura, H.; Nishioka, I. *J. Chem. Soc., Perkin Trans. 1* **1985**, 1163−172.
(19) Tanaka, T.; Kirihara, S.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1993**, *41*, 1708−1716.
(20) This signal was broadened by dynamic rotational isomerism of the C−C bond between the glucose C-1 and the gallic acid methyl ester moiety. Similar phenomena were observed in the case of **9** and **10**.
(21) Okuda, T.; Yoshida, T.; Hatano, T.; Koga, T.; Toh, N.; Kuriyama, K. *Tetrahedron Lett.* **1982**, *23*, 3937−3940.
(22) Schmidt, O. T.; Ebert, W.; Kopp, M. *Justus Liebigs Ann. Chem.* **1969**, *729*, 251−254.
(23) Okuda, T.; Yoshida, T.; Hatano, T. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Springer-Verlag: New York, 1995; Vol. 66, pp 2−117.
(24) Nishizawa, M.; Yamagishi, T.; Nonaka, G.; Nishioka, I. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2963−2968.
(25) *tert*-Butyl methyl ether was used in place of diethyl ether for environmental reasons. The recovery of this solvent on evaporation with a rotary evaporator was much higher than diethyl ether.
(26) Tanaka, T.; Nonaka, G.; Nishioka, I. *Phytochemistry* **1983**, *22*, 2575−2578.

NP020608G