Structure and Biogenesis of Jolkinin, a Highly Oxygenated Ellagitannin from *Euphorbia jolkinii*

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A new ellagitannin, jolkinin (**2**), was isolated from the fresh whole plant of *Euphorbia jolkinii*, and its structure, including absolute configuration, was determined on the basis of spectroscopic and chemical evidence. A highly oxygenated acyl group with unique hexacyclic structure is attached to the 2,4-positions of 1-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-*β*-D-glucopyranose. The structural similarity to elaeocarpusin (**4**) suggests that jolkinin is produced by a condensation reaction between ascorbic acid and geraniin (**1**), the dominant ellagitannin in this plant, which contains a dehydrohexahydroxydiphenoyl group. A plausible mechanism for jolkinin formation is also proposed.

Recently, various health benefits of polyphenols in plants have attracted much scientific interest. Among the polyphenols, tannins are a group of compounds that bind to proteins by hydrophobic, electrostatic, and ionic interactions;[1] therefore, tannins inhibit digestive enzymes[2,3] and are believed to act as defensive substances against microorganisms[4] and herbivores.[5,6] Ellagitannins are defined as a group of tannins having hexahydroxydiphenic acid (HHDP) esters in the molecule, and most ellagitannins have a D-glucose polyalcohol core.[7] When a HHDP ester bridges the C-3 and C-6 or the C-1 and C-6 positions of the D-glucopyranose, the pyranose ring adopts a $^1C_4$ conformation, in which the oxygen atoms and the C-6 methylene carbon are fixed in axial orientations. In such ellagitannins, some oxidized forms of the HHDP esters are often found at the glucose 2,4-positions. The most typical one is geraniin (**1**) (Figure 1),[8] which occurs in many Euphorbiaceous plants as a major phenolic constituent. *Euphorbia jolkinii* Boiss. (Euphorbiaceae) also contains **1** as a major ellagitannin (0.098% from fresh whole plant), along with several related compounds.[9] In our continuing chemical studies on tannins in Euphorbiaceous plants, a new ellagitannin named jolkinin (**2**) was isolated from *E. jolkinii*. This paper deals with the structural determination of this ellagitannin.

Results and Discussion

Fresh whole *E. jolkinii* plants were extracted with 80% aqueous acetone, and the extract was first fractionated by Sephadex LH-20 column chromatography. Then each fraction was separated by repeated column chromatography over Sephadex LH-20, MCI-gel CHP20P, and Bondapak C_{18}/Porasil B to give jolkinin (**2**) (0.002%), together with 1-*O*-,[9] 1,6-di-*O*-,[9] 1,2,3,6-tetra-*O*-,[9] 1,3,4,6-tetra-*O*-,[9] 2,3,4,6,-tetra-*O*-,[10] and 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose, corilagin (**3**),[9,11] geraniin (**1**),[8,9] furosin,[12] putranjivain A,[13] carpinusin,[9,14] helioscopinins A and B,[9,15] and jolkianin.[9] The known compounds were identified by direct comparison of spectroscopic and physical data.

Jolkinin was obtained as a tan amorphous powder and characterized as an ellagitannin by the standard color reactions with ferric chloride (dark blue) and sodium nitrite−acetic acid (reddish brown).[16] The ^{1}H and ^{13}C NMR spectra exhibited signals due to a fully acylated hexopyranose along with aromatic and carboxyl carbon signals, which is consistent with **2** being a hydrolyzable tannin. The presence of galloyl and HHDP esters was suggested by the ^{1}H and ^{13}C NMR signals listed in the Experimental Section, and hydrolysis of **2** in hot H_2O to yield corilagin [1-*O*-galloyl-3,6-(*R*)-HHDP-*β*-D-glucopyranose (**3**), Figure 1] confirmed the partial structure. This result showed not only the location of the galloyl and HHDP esters on the *β*-D-glucopyranose ring but also *R*-atropisomerism of the HHDP biphenyl bond.[8,17]

The remaining part of the molecule was comprised of an aromatic ring (C-1″−C-6″), three carboxyl (C-7, C-1′, and C-7″), seven aliphatic quaternary (C-2, C-4, C-5, C-6, C-2′, C-3′, and C-4′), and three methine (C-1, C-3, and C-5′) carbons, and one methylene (C-6′) carbon. Chemical shifts of the aromatic carbon signals showed that the aromatic ring is a pyrogallol ring. In the HMBC spectrum, an aromatic singlet (δ 7.01, H-3″) of this pyrogallol ring was correlated to a carboxyl carbon signal at δ 165.4 (C-7″), indicating that the C-7″ carboxyl group is at C-2″. Three of the aromatic carbons (C-1″, C-2″, and C-6″) were correlated to a benzyl methine proton (δ 4.86, H-1), which was coupled with two acetal carbons (C-5 and C-6), two quaternary carbons (C-2 and C-2′), and a methine carbon (C-3). In addition, correlations of the C-3 methine proton signal (δ 2.64, H-3) with the C-2, C-5, and another acetal carbon (C-4) signal suggested that the three acetal carbons (C-4, C-5, and C-6), two methine carbons (C-1 and C-3), and a quaternary carbon (C-2) formed a cyclohexane ring. This was supported by ^{1}H−^{1}H long-range coupling between H-1 and H-3 observed in the ^{1}H−^{1}H COSY spectrum. Furthermore, the HMBC correlations of the C-7 carboxyl carbon with H-1 and H-3 indicated that this group was attached to C-2. The ^{1}H−^{1}H COSY spectrum revealed linkage between oxygen-bearing methine (C-5′) and methylene (C-6′) carbons. In the HMBC spectrum, H-6′ correlated with C-4′ and C-5′, and H-5′ correlated with C-4′,

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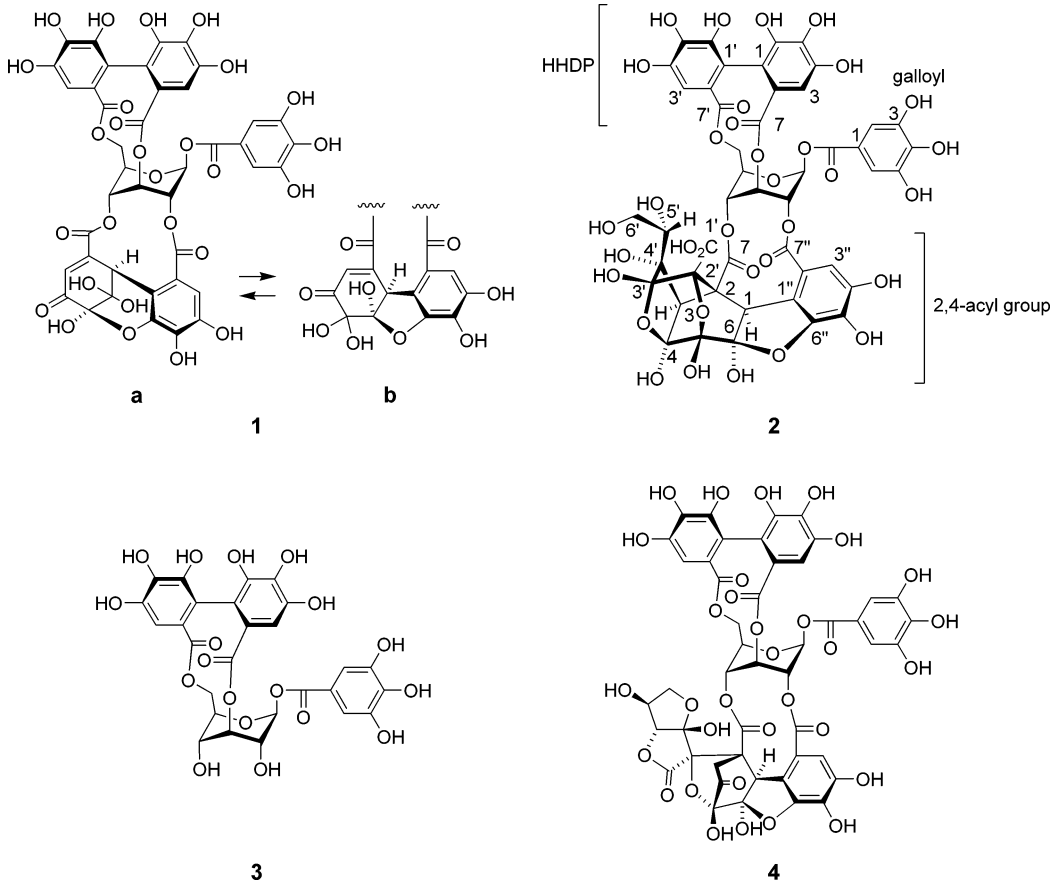


Figure 1. Structures of compounds **1**−**4**.

C-3′, C-2′, C-6′, and C-3. The aforementioned H-3 was correlated with C-4′, C-3′, and C-2′, and C-2′ was also coupled with H-1. From these correlations, it was deduced that C-2′ and C-4′ of the carbon chain C-2′ through C-6′ were connected to the C-2 and C-3 positions of the above-mentioned cyclohexane ring. Consequently, a cyclopentane ring was formed by C-2, C-3, C-4′, C-3′, and C-2′, as shown in Figure 1. The remaining carboxyl carbon C-1′ was presumed to be attached to C-2′, on the basis of observation of a small ^4J correlation peak with H-3 in the HMBC spectrum. The ester carbonyl carbons C-7 and C-7″ of this acyl group showed HMBC cross-peaks with the glucose H-4 and H-2, respectively, confirming the connection of this acyl group to the glucopyranose 2,4-positions.

The negative ion FABMS of **2** exhibited the $[M - H]^-$ peak at *m*/*z* 1143. Taking the above-mentioned chemical and spectral data into account, the molecular formula of **2** is $C_{47}H_{36}O_{34}$, which was also supported by elemental analysis. The index of unsaturation calculated from the formula is 30, and 25 degrees of unsaturation were accounted for by three carboxyl groups; the aromatic ring of the 2,4-acyl group, the corilagin moiety, and a macrocyclic lactone ring formed between these two parts. The remaining 5 degrees of unsaturation indicated that there are five ring structures in the aliphatic part of the 2,4-acyl group. Of the five rings, two were accounted for by the above-mentioned cyclohexane and cyclopentane rings. Comparison of the chemical shifts of the C-6 (δ 106.0) and C-6″ (δ 149.9) of **2** with those of the two equilibrium forms of the dehydrohexahydroxydiphenoyl (DHHDP) esters of geraniin (**1**) indicated that the chemical shifts were similar to those of the five-membered ring hemiacetal form [**1b**: δ 108.9 (C-6) and 147.3 (C-6″)] and different from those of the six-membered ring form [**1a**: δ 92.5(C-6) and 143.4 (C-6″)];[8] therefore, formation of an ether linkage between C-6 and C-6″ was deduced. The remaining two rings were presumed to be formed by ether linkages between the oxygen-bearing quaternary carbon C-2′ and the acetal carbon C-5 and between two acetal carbons C-3′ and C-4.

To obtain further evidence for the structure, methylation of **2** was attempted. However, under usual conditions using $(CH_3)_2SO_4$ or CH_2N_2, **2** gave a complex mixture. Therefore, the acetonide of **2** was prepared prior to methylation, because aliphatic vicinal diols were expected to be present in the acyl group. Methylation of the acetonide successfully yielded two products, **2a** and **2b**. The molecular mass of **2b** [*m*/*z* 1376] was 14 mass units larger than that of **2a** [*m*/*z* 1362], which corresponds to the mass of a methyl group. The ^{1}H NMR spectra of **2a** and **2b** (see Experimental Section) were closely related to each other; however, the spectrum of **2a** showed a sharp singlet signal due to a hydroxyl proton at δ 5.88, which showed a ^2J HMBC correlation with the carbon signal attributable to the C-3′ acetal carbon. An additional methyl proton signal was observed at δ 3.47 in the spectrum of **2b**, and this signal showed a correlation peak with C-3′ in the HMBC spectrum. These spectral differences indicated that **2b** was a methyl ether with an additional methyl group at the C-3′ acetal hydroxyl group of **2a**.

The ^{13}C NMR spectrum of **2a** exhibited a signal attributable to a ketone carbon (C-4), which was not observed in the spectrum of **2**. The ketone signal showed a cross-peak with the H-3 of the acyl group in the HMBC spectrum; therefore, the ketone in **2a** was assigned to the C-4 position. In addition, one of the H-6′ methylene protons of **2a** showed a correlation peak with the C-3′ acetal carbon. Furthermore, the C-6′ carbon signal appeared at a lower field (δ 74.3) than that of **2** (δ 62.7). From these findings, it was

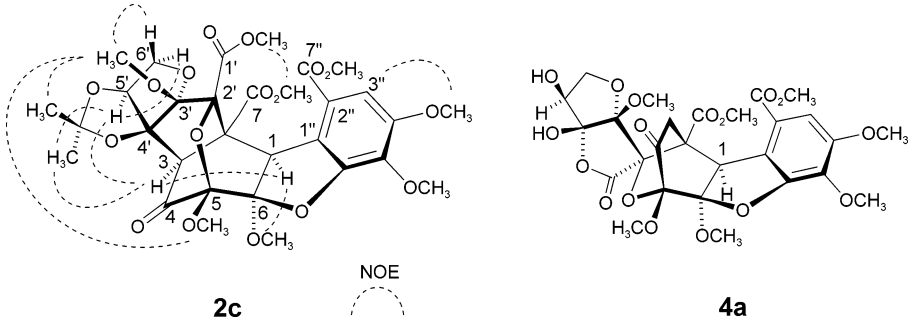
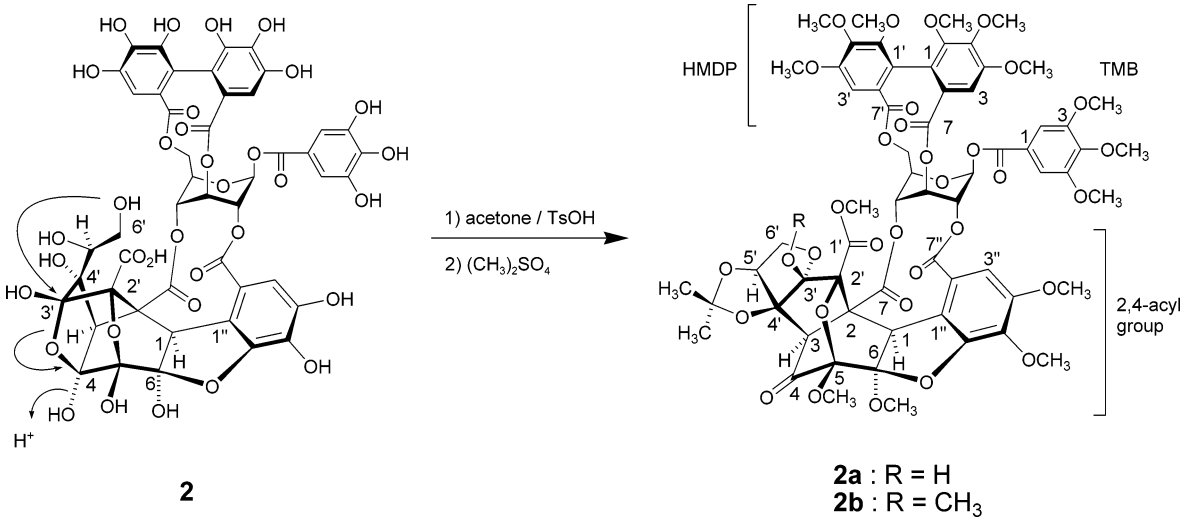


Figure 2. Structures of **1c** and **4a** and important NOESY correlations for **1c**.

Scheme 1. Acetonide Formation Followed by Methylation



deduced that formation of a new ether linkage between the acetal C-3′ and the C-6′ hydroxyl group, which was accompanied by cleavage of the ether linkage between C-4 and C-3′ in **2**, occurred during derivation of **2a** from **2** (Scheme 1). The acetonide moieties were presumed to be attached to the vicinal hydroxyl groups at the C-5′ and C-4′ positions, on the basis of the HMBC correlation between H-5′ and a quaternary carbon of the acetonide. These HMBC correlations described for **2a** were similarly observed in the spectrum of **2b**, and other HMBC correlations observed for **2a** and **2b** were consistent with the formulation of the structures of **2a** and **2b** shown in Scheme 1 and also supported the carbon skeleton of the acyl group of **2**. The HMBC correlations of the methoxyl groups in **2a** indicated that the C-1′ of **2** was a free carboxyl group and the C-5 and C-6 were hemiacetal carbons.

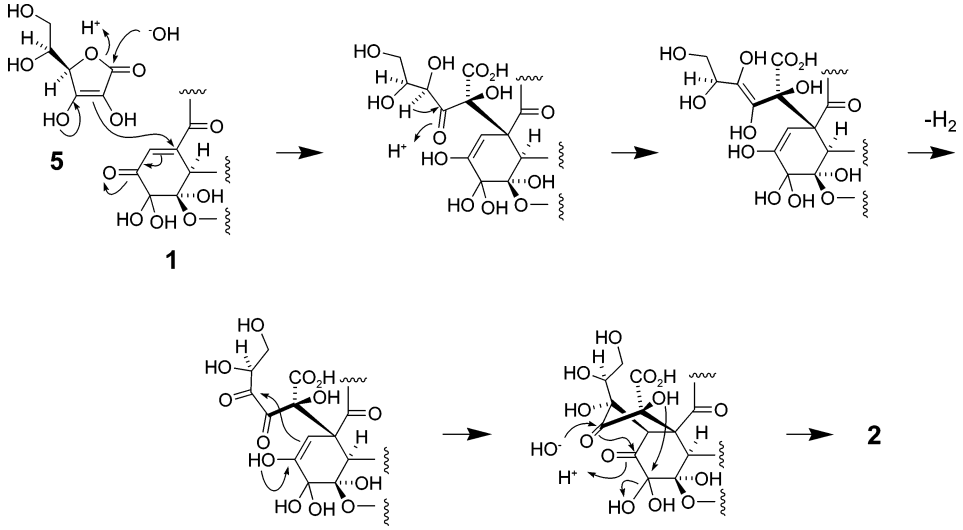
The relative stereochemistry of the acyl group attached to the glucose 2,4-positions was determined by observation of NOEs for methanolysate **2c** (Figure 2), which was obtained along with methyl 3,4,5-trimethoxybenzoate and dimethyl (*R*)-4,4′,5,5′,6,6′-hexamethoxydiphenate.[18] One-dimensional differential NOE experiments showed NOEs of H-3 with H-1 and H-5′, indicating that these protons are located on the same side of the molecule. In addition, two-dimensional NOESY correlations of three acetal methyl groups, two acetonide methyls, and C-6′ methylene protons shown in Figure 2 also supported the configuration of **2c**. The absolute configuration of **2c** was determined by comparison of the CD spectrum with that of **4a**, which was derived from elaeocarpusin (**4**) by methylation followed by methanolysis.[19] The CD spectrum of **2c** showed a negative Cotton effect at 260 nm and a positive Cotton effect at 230 nm, which was similar to that of **4a** (negative Cotton at 263 nm and positive Cotton at 229 nm), where the C-1 benzyl methine has an *R*-configuration. Accordingly, the structure of jolkinin was concluded to be as shown in formula **2**.

Elaeocarpusin (**4**) and related ellagitannins were previously isolated from various Euphorbiaceous[15,20] and Elaeocarpaceous plants,[19] and it was shown that the elaeocarpusinoyl group, the acyl group attached to the glucose 2,4-positions in **4**, was derived by addition of L-ascorbic acid (**5**) to the (*R*)-DHHDP group.[20] The structural similarity to **4** suggested that **2** was also derived from ascorbic acid and geraniin (**1**), which is the dominant ellagitannin of *E. jolkinii*. However, **4** was not isolated from this plant. Plausible biogenesis of **2** from **1** is demonstrated in Scheme 2, where **5** reacts from the β side of the DHHDP group, and the pathway includes an oxidation step. In contrast, in the biosynthesis of **4** from **1**, **5** is attacked from the α side of the DHHDP group. Hence, jolkinin represents a new type of ascorbic acid adduct of ellagitannins and could be important from the viewpoint of ellagitannin metabolism in the plant kingdom.

Experimental Section

General Experimental Procedures. Optical rotations were measured with JASCO DIP-4 and DIP-370 digital polarimeters. IR spectra were obtained with a JASCO FT/IR-410 spectrophotometer. CD spectra were measured by using a JASCO J-600 spectropolarimeter. ^{1}H, ^{13}C NMR, ^{1}H−^{1}H COSY, NOESY, HSQC, and HMBC spectra for **2**, **2a**, and **2b** were recorded with a Unity plus 500 spectrometer (Varian Inc.) operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. NMR spectra for **2c** were measured by using a JEOL GX-270 spectrometer operating at 270 MHz for ^{1}H and 67.5 MHz for ^{13}C. FABMS spectra were recorded by using a JMS DX-303 spectrometer (JEOL Ltd., Japan), using *m*-nitrobenzyl alcohol

Scheme 2. Plausible Biogenesis of **1** from **3** and Ascorbic Acid



or glycerol as the matrix. Elemental analysis was carried out with a Perkin-Elmer 2400 II analyzer (Perkin-Elmer, Inc.). Column chromatography was done on MCI-gel CHP 20P (Mitsubishi Chemical Co.), Bondapak C_{18}/Porasil B (37−75 μm, Waters Associates, Inc.), Sephadex LH-20 (Pharmacia Fine Chemical Co.), and silica gel 60 (70−230 mesh, Merck). Thin-layer chromatography (TLC) for tannins was performed on precoated Kieselgel 60 F_{254} plates, 0.2 mm thick (Merck), with benzene−ethyl formate−formic acid (1:7:1, v/v), or cellulose F_{254} plates, 0.1 mm thick (Merck), with 2% acetic acid. Spots were detected by UV illumination and sprayed with 2% ethanolic $FeCl_3$ or 10% sulfuric acid reagent followed by heating.

Plant Material. Whole plants of *Euphorbia jolkinii* Boiss. were collected in Yobuko, Saga Prefecture, in April 1989. The voucher specimen (KPMG 0427-89/4) was deposited at the Herbarium, Faculty of Pharmaceutical Sciences, Kyushu University.

Isolation. The plant material and extraction procedures were described in our previous paper.[9] Fraction 2 (200 g), obtained from the aqueous acetone extract of fresh whole plants (50 kg) by Sephadex LH-20 column chromatography (H_2O−MeOH), was further separated by MCI-gel CHP20P column chromatography with H_2O containing increasing amounts of MeOH, to give fractions 2-1 (15 g), 2-2 (150 g), and 2-3 (25 g). Repeated chromatography of fraction 2-1 on Sephadex LH-20 (60% MeOH), Bondapak C_{18}/Porasil B (H_2O−MeOH), and MCI-gel CHP20P (H_2O−MeOH) yielded carpinusin (1.3 g). Separation of fraction 2-2 by column chromatography over Sephadex LH-20 (60−100% MeOH) and MCI-gel CHP20P (H_2O−MeOH) afforded **1** (52 g), **2** (1.4 g), and **3** (0.9 g). On similar chromatography, fraction 2-3 yielded 1,3,4,6-tetra-*O*-galloyl-D-glucopyranose (18 mg), putranjivain A (40 mg), helioscopinin B (2.2 g), and helioscopinin A (3.2 g).

Jolkinin (2): tan amorphous powder; $[\alpha]^{28}_D$ −55.4° (*c* 0.7, acetone); IR ν_{max} (neat) 3448, 1719, 1614, 1526, 1446 1316, 1213, and 1036 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) glucose, δ 6.41 (1H, br s, H-1), 5.27 (1H, br s, H-2), 6.21 (1H, br t, J = 3.0 Hz, H-3), 5.18 (1H, br d, J = 3.0 Hz, H-4), 4.78 (2H, m, H-5, H-6), 4.38 (1H, m, H-6); galloyl, δ 7.15 (2H, s, H-2, 6); HHDP, δ 7.06 (1H, s, H-3), 6.63 (1H, d, H-3′); 2,4-acyl group, δ 4.86 (1H, s, H-1), 2.64 (1H, s, H-3), 4.31 (1H, dd, J = 3.9, 4.2 Hz, H-5′), 3.79 (1H, dd, J = 4.2, 11.6 Hz, H-6′), 3.67 (1H, dd, J = 3.9, 11.6 Hz, H-6′), 7.01 (1H, s, H-3″); ^{13}C NMR (500 MHz, $CDCl_3$) glucose, δ 92.1 (C-1), 70.6 (C-2), 60.8 (C-3), 68.6 (C-4), 72.4 (C-5), 64.0 (C-6); galloyl, δ 120.0 (C-1), 110.5 (C-2, 6), 145.9 (C-3, 5), 139.8 (C-4), 165.3 (C-7); HHDP, δ 117.0 (C-1), 115.0 (C-1′), 124.2 (C-2), 125.7 (C-2′), 110.5 (C-3), 107.5 (C-3′), 144.6 (C-4), 145.3 (C-4′), 137.7 (C-5), 136.2 (C-5′), 145.1 (C-6), 144.9 (C-6′), 166.4 (C-7), 168.7 (C-7′); 2,4-acyl group, δ 53.2 (C-1), 54.9 (C-2), 56.5 (C-3), 102.4 (C-4), 96.2 (C-5), 106.0 (C-6), 169.2 (C-7), 173.2(C-1′), 86.6 (C-2′), 104.8 (C-3′), 86.9 (C-4′), 81.5 (C-5′), 62.7 (C-6′), 118.5 (C-1″), 115.3 (C-2″), 112.9 (C-3″), 147.3 (C-4″), 134.8 (C-5″), 149.9 (C-6″), 165.4 (C-7″); HMBC correlations (H/C) glc-1/galloyl-7, glc-2, 3, 5; glc-2/acyl-7″, glc-1, 3, 4; glc-3/HHDP-7, glc-1, 2, 4, 5; glc-4/acyl-7, glc-2, 3; glc-5/glc-6; glc-6/HHDP-7′, glc-4; galloyl-2,6/galloyl-1, 3, 4, 6, 7; HHDP-3/HHDP-1, 2, 4, 5, 7; HHDP-3′/HHDP-1′, 4′, 5′, 7′; 2, 4-acyl-1/2, 4-acyl-2, 3, 5, 6, 7, 2′, 1″, 2″, 6″, 7″(^4J); 2, 4-acyl-3/2, 4-acyl-2, 4, 5, 7, 1′(^4J), 2′, 3′, 4′; 2, 4-acyl-5′/2, 4-acyl-3, 4, 3′, 4′, 6′; 2, 4-acyl-6/2, 4-acyl-4′, 5′; 2, 4-acyl-3″/2, 4-acyl-1(^4J), 1″, 2″, 4″, 5″, 6″(^4J), 7″; FABMS *m*/*z* 1143 [M − H]$^-$; *anal*. calcd for $C_{47}H_{36}O_{34}$ H_2O C, 48.55; H, 3.29; found C, 48.31; H, 3.19.

Partial Hydrolysis of 2. A solution of **2** in H_2O (8 mg/mL) was heated at 90 °C for 12 h. The mixture was subjected to MCI-gel CHP20P column chromatography (1 cm i.d. × 7 cm) with H_2O containing increasing proportions of MeOH, to yield gallic acid (1.5 mg) and corilagin (1.7 mg). The latter was identified by comparison of the ^{1}H NMR spectrum with that of an authentic sample.[9]

Preparation of Methyl Ethers 2a and 2b. A solution of **2** (300 mg) and *p*-toluenesulfonic acid (10 mg) in acetone (20 mL) was heated under reflux for 2 h. After concentration, the mixture was chromatographed on a Sephadex LH-20 column (1.5 cm i.d. × 15 cm) with H_2O containing increasing proportions of MeOH to give an acetonide (250 mg). The acetonide was methylated by $(CH_3)_2SO_4$ (2 mL), with K_2CO_3 (2 g) in acetone (30 mL) under reflux for 2 h. The products were separated by silica gel column chromatography (hexane−acetone, 1:1) to give **2a** (58.6 mg) and **2b** (72.0 mg).

Methyl Ether 2a: white amorphous powder; $[\alpha]^{28}_D$ −102.6° (*c* 0.2, $CHCl_3$), IR ν_{max} (neat) 3450, 2943, 2841, 1750, 1719, 1589, and 1459 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) trimethoxybenzoyl (TMB), δ 7.21 (2H, s, H-2,6), 3.68 (6H, s, C-3, 5-OCH_3); hexamethoxydiphenoyl (HMDP), δ 6.66 (1H, s, H-3), 6.72 (1H, s, H-3′), 4.00, 3.98, 3.97, 3.95, 3.72, 3.70, 3.25 (each 3H, s, OCH_3); glucose, δ 6.62 (1H, br s, H-1), 5.35 (1H, br s, H-2), 6.03 (1H, ddd, J = 1.4, 2.3, 5.0 Hz, H-3), 5.47 (1H, br d, J = 5 Hz, H-4), 4.78 (1H, br dd, J = 8.5, 11.1 Hz, H-5), 5.01 (1H, t, J = 11.1 Hz, H-6), 4.42 (1H, dd, J = 8.5, 11.1 Hz, H-6); 2, 4-acyl group, δ 5.38 (1H, d, J = 0.5 Hz, H-1), 3.64 (1H, d, J = 0.5 Hz, H-3), 4.42 (1H, d, J = 4.2 Hz, H-5′), 4.45 (1H, dd, J = 4.2, 10.1 Hz, H-6′), 4.13 (1H, d, J = 10.1 Hz, H-6′), 7.47 (1H, s, H-3″), 1.54 (3H, s, acetonide-H-2), 1.42 (3H, s, acetonide-H-3), 5.88 (1H, s, C-3′-OH), 3.88 (3H, s, C-5-OCH_3), 3.68 (3H, s, C-6-OCH_3), 4.08 (3H, s, C-1′-OCH_3), 3.86 (3H, s, C-4″-OCH_3), 3.97 (3H, s, C-5″-OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) TMB, δ 122.9 (C-1), 106.9 (C-2, 6), 153.3 (C-3, 5), 143.3 (C-4), 164.1 (C-7); HMDP, δ 124.5, 120.9 (C-1,1′), 128.1, 125.7 (C-2,2′), 107.5 (C-3), 104.9 (C-3′), 153.4, 152.6, 152.6, 152.3 (C-4, 4′, 6, 6′), 145.45, 144.02 (C-5, 5′), 164.59 (C-7), 167.50 (C-7′); glucose, δ 91.8 (C-1), 70.3 (C-2), 59.9 (C-3), 67.0 (C-4), 70.5 (C-5), 63.1 (C-6); 2,

4-acyl group, δ 47.8 (C-1), 66.4 (C-2), 59.5 (C-3), 206.0 (C-4), 96.0 (C-5), 101.9 (C-6), 166.1 (C-7), 170.5 (C-1′), 92.6 (C-2′), 111.1 (C-3′), 94.6 (C-4′), 82.8 (C-5′), 74.3 (C-6′), 110.6 (C-1″), 124.3 (C-2″), 113.8 (C-3″), 152.9 (C-4″), 142.4 (C-5″), 147.2 (C-6″), 165.6 (C-7″), 114.1 (acetonide-C-1), 26.4 (acetonide-C-2), 26.0 (acetonide-C-3), 52.0 (C-5-OCH_3), 56.4 (C-6-OCH_3), 53.4 (C-1′-OCH_3), 56.1 (C-4″-OCH_3); OCH_3, δ 61.1, 60.9, 60.8, 60.73, 60.70, 60.6, 56.4, 56.0, 55.2; HMBC correlations (H/C) 2, 4-acyl-1/2, 4-acyl-2, 3, 5, 6, 7, 2′, 4′(^4J), 1″, 2″, 6″; 2, 4-acyl-3/2, 4-acyl-2, 4, 5, 7, 1′(^4J), 2′, 3′, 4′, 5′; 2, 4-acyl-5′/2, 4-acyl-3, 3′, 6′, acetonide-1; 2, 4-acyl-6′/2, 4-acyl-3′, 4′, 5′; 2, 4-acyl-3″/2, 4-acyl-1(^4J), 1″, 2″, 4″, 5″, 6″(^4J), 7″; 2, 4-acyl-3′-OH/2, 4-acyl-1′(^4J), 2′, 3′, 4′; 2, 4-acyl-5-OCH_3/2, 4-acyl-5; 2, 4-acyl-6-OCH_3/2, 4-acyl-6; 2, 4-acyl-1′-OCH_3/2, 4-acyl-1′, 2′(^4J); 2, 4-acyl-4″-OCH_3/2, 4-acyl-4″; acetonide-2, 3/acetonide-1; FABMS *m*/*z* 1362 M^+; *anal*. calcd for $C_{64}H_{66}O_{33}$ C, 56.39; H, 4.88; found C, 56.14; H, 4.88.

Methyl Ether 2b: white amorphous powder; $[\alpha]^{28}_D$ −28.2° (*c* 0.4, $CHCl_3$); IR ν_{max} (neat) 2945, 2848, 1751, 1718, 1592, and 1458 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) TMB, δ 7.21 (2H, s, H-2, 6), 3.69 (6H, s, 3,5-OCH_3); HMDP, δ 6.68 (1H, s, H-3), 6.73 (1H, s, H-3′), 3.84 (3H, s, 4′-OCH_3), 3.21 (3H, s, 4-OCH_3); glucose, δ 6.59 (1H, br s, H-1), 5.29 (1H, br s, H-2), 6.32 (1H, ddd, J = 1.1, 2.5, 5.0 Hz, H-3), 5.12 (1H, br d, J = 5 Hz, H-4), 4.60 (1H, br dd, J = 8.2, 10.8 Hz, H-5), 5.03 (1H, t, J = 10.8 Hz, H-6), 4.48 (1H, dd, J = 8.2, 10.8 Hz, H-6); 2, 4-acyl group: δ 5.59 (1H, d, J = 0.4 Hz, H-1), 3.15 (1H, d, J = 0.4 Hz, H-3), 4.51 (1H, dd, J = 2.1, 3.9 Hz, H-5′), 4.26 (2H, br s, H-6′), 7.18 (1H, s, H-7″), 1.56 (3H, s, acetonide-H-2), 1.36 (3H, s, acetonide-H-3), 3.67 (3H, s, C-5-OCH_3), 3.47 (3H, s, C-6-OCH_3), 3.47 (3H, s, C-3′-OCH_3), 3.89 (3H, s, C-4″-OCH_3); OCH_3: δ 4.00, 3.98, 3.973, 3.971, 3.96, 3.75, 3.71 (each 3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) TMB, δ 122.9 (C-1), 106.9 (C-2, 6), 153.3 (C-3, 5), 143.3 (C-4), 164.1 (C-7); HMDP, δ 124.2, 121.8 (C-1, 1′), 128.4, 125.8 (C-2, 2′), 107.7 (C-3), 104.3 (C-3′), 153.8, 152.7, 152.4, 152.3 (C-4, 4′, 6, 6′), 145.4, 144.1 (C-5,5′), 164.6 (C-7), 167.4 (C-7′); glucose, δ 92.6 (C-1), 69.1 (C-2), 60.4 (C-3), 66.9 (C-4), 72.2 (C-5), 63.5 (C-6); 2, 4-acyl group, δ 45.3 (C-1), 56.7 (C-2), 58.5 (C-3), 198.8 (C-4), 98.4 (C-5), 110.3 (C-6), 165.1 (C-7), 165.5 (C-1′), 88.0 (C-2′), 114.1 (C-3′), 92.6 (C-4′), 87.8 (C-5′), 74.0 (C-6′), 116.3 (C-1″), 120.8 (C-2″), 109.7 (C-3″), 152.8 (C-4″), 137.1 (C-5″), 150.7 (C-6″), 164.2 (C-7″), 114.3 (acetonide-C-1), 25.7 (acetonide-C-2), 24.2 (acetonide-C-3), 54.0 (C-5-OCH_3), 50.3 (C-6-OCH_3), 52.3 (C-1′-OCH_3), 52.4 (C-3′-OCH_3), 56.5 (C-4″-OCH_3); OCH_3, δ 61.1, 60.83, 60.79, 60.75, 60.70, 60.69, 56.5, 56.0, 55.2; HMBC correlations (H/C) 2, 4-acyl-1/2, 4-acyl-2, 3, 5, 6, 7, 2′, 1′(^4J), 2′, 4′(^4J), 1″, 2″, 6″, 7″(^4J); 2, 4-acyl-3/2, 4-acyl-2, 4, 5, 7, 1′(^4J), 2′, 3′, 4′, 5′; 2, 4-acyl-5′/2, 4-acyl-3, 3′, 6′, acetonide-1; 2, 4-acyl-6′/2, 4-acyl-3′, 4′, 5′; 2, 4-acyl-3″/2, 4-acyl-1(^4J), 1″, 2″, 4″, 5″, 6″(^4J), 7″; 2, 4-acyl-5-OCH_3/2, 4-acyl-5; 2, 4-acyl-6-OCH_3/2, 4-acyl-6; 2, 4-acyl-1′-OCH_3/2, 4-acyl-1′, 2′(^4J); 2, 4-acyl-4″-OCH_3/2, 4-acyl-4″; acetonide-2, 3/acetonide-1; FABMS *m*/*z* 1376 M^+; *anal*. calcd for $C_{65}H_{68}O_{33}$ H_2O C, 55.96; H, 5.06; found C, 55.78; H, 4.92.

Methanolysis of 2b. A solution **2b** (40 mg) in MeOH (1 mL) and 10% aqueous NaOH (2 mL) was heated at 70 °C for 1 h. The mixture was acidified with 1 M HCl and extracted with ether. The ether layer was dried over Na_2SO_4 and concentrated. The residue was dissolved in MeOH (2 mL) and treated with CH_2N_2 ether solution at 0 °C for 2 h. The products were separated by silica gel column chromatography (toluene−acetone, 93:7) to yield methyl trimethoxybenzoate (3 mg), dimethyl (*R*)-hexamethoxydiphenate (8 mg), and **2c** (10 mg): white amorphous powder; $[\alpha]^{27}_D$ 6.2° (*c* 0.8, acetone); IR ν_{max} (neat) 2951, 2843, 1747, 1720, 1610, and 1510 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.20 (1H, s, H-3″), 5.51 (1H, s, H-1), 4.89 (1H, dd, J = 2, 6 Hz, H-5′), 4.20 (1H, dd, J = 2, 11 Hz, H-6′), 4.11 (1H, dd, J = 6, 11 Hz, H-6′), 3.98 (3H, s, 5″-OCH_3), 3.93 (3H, s, 7″-OCH_3), 3.91 (3H, s, 4″-OCH_3), 3.83 (3H, s, 7-OCH_3), 3.67 (3H, s, 5-OCH_3), 3.51 (3H, s, 1′-OCH_3), 3.50 (3H, s, 3′-OCH_3), 3.41 (3H, s, 6-OCH_3), 2.97 (1H, s, H-3), 1.46, 1.29 (each 1H, s, acetonide CH_3); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 198.9 (C-4), 166.1, 166.0, 165.9 (C-7, 1′, 7″), 152.3 (C-4″), 149.9 (C-6″), 137.0 (C-5″), 123.0 (C-2″), 119.2 (C-1″), 114.8 (acetonide C), 112.3 (C-3′), 110.2 (C-6), 107.9 (C-3″), 99.0 (C-5), 94.4 (C-4′), 87.1 (C-2′), 79.3 (C-5′), 74.7 (C-6′), 58.1 (C-2), 57.4 (C-3), 60.8 (5″-OCH_3), 56.5 (4″-OCH_3), 53.2 (5-OCH_3), 52.5 (7″-OCH_3), 52.1 (×3, 6-OCH_3, 7-OCH_3, 1′-OCH_3), 51.4 (3′-OCH_3), 47.6 (C-1), 25.9, 25.3 (acetonide CH_3); CD (1.5 × 10^{-5} M, EtOH) $\Delta\epsilon$ (nm), −0.35 (260), 11.5(230); FABMS *m*/*z* 681 $[M + H]^+$; *anal*. calcd for $C_{31}H_{36}O_{17}$ C, 54.70; H, 5.29; found C, 54.89; H, 5.53.

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