Rubusuaviins A—F, Monomeric and Oligomeric Ellagitannins from Chinese Sweet Tea and Their α-Amylase Inhibitory Activity

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Six new ellagitannins herein, rubusuaviins A—F, were isolated from the aqueous acetone extract of Chinese sweet tea (Tien-cha, dried leaves of *Rubus suavissimus* S. Lee) together with seven known tannins. Rubusuaviin A was characterized as 1-*O*-galloyl-2,3-*O*-(*S*)-HHDP-4,6-*O*-(*S*)-sanguisorboyl-β-D-glucopyranose. Rubusuaviins B, C, and E are dimeric, trimeric, and tetrameric ellagitannins, respectively, in which the sanguisorboyl groups were connected ellagitannin units. Rubusuaviins D and F were desgalloyl derivatives of rubusuaviins C and E, respectively. The inhibition of α-amylase activity by rubusuaviins and related ellagitannins was compared. Ellagitannins with β-galloyl groups at the glucose C-1 positions showed stronger inhibition compared with the α-galloyl and desgalloyl compounds. The molecular weight of these compounds was not important for the inhibition of α-amylase activity.

Key words *Rubus suavissimus*; Rosaceae; ellagitannin; amylase; polyphenol; tannin

Rubus suavissimus S. Lee (Rosaceae) is a shrub widely grown in Guang-Xi province, China. The leaves are used as an herbal tea called Tien-cha (sweet tea) or as natural sweeteners. The sweetening principle, which is 114-fold sweeter than sucrose, is a diterpene glycoside called rubusoside (**18**).[1,2] In addition, the so-called GOD-type ellagitannins in this plant were reported to have antiallergy or antiinflammatory activities.[3] The GOD-type ellagitannins are a group of tannins with a sanguisorboyl ester, a characteristic acyl group found in *Rubus* and *Sanguisorba* species.[4—8] Sanguiins, which are monomeric–oligomeric compounds isolated from *Sanguisorba officinalis* L., are the first isolated and typical examples of ellagitannins. Ellagitannins strongly inhibit NO production *in vivo* and contribute to improved renal function by reversing the elevated levels of blood urea nitrogen and creatinine caused by $ONOO^-$.[9—11] Lambertianins, isolated from *Rubus lambertianus*, are closely related to sanguiins. However, these tannins differ in configuration at anomeric positions of the component glucose moieties.[4] In these dimeric and oligomeric tannins, the sanguisorboyl group is connected to two ellagitannin monomers to form dimeric and oligomeric ellagitannins, and therefore it is deduced that the sanguisorboyl group is produced by intermolecular regioselective oxidative coupling between a 3,3′,4,4′,5,5′-hexahydroxydiphenoyl (HHDP) group and a galloyl group.[12] The ellagitannins of *R. suavissimus* are considered to be similar to sanguiins and lambertianins, but the chemical details have not been clarified. This paper describes the isolation and elucidation of the structure of GOD-type ellagitannins and their α-amylase inhibitory activity.

Results and Discussion

Dried leaves of *R. suavissimus* collected in Guang-Xi, China, were homogenized in 70% acetone. The extract was concentrated and dried with a spray dryer. Fractionation of the extract on Sepabeads SP825 column chromatography afforded tannin fractions and diterpene glycoside fractions. Normal-phase HPLC of the tannin fractions indicated the presence of monomeric to pentameric hydrolysable tannins (Fig. 1).[7,8] The tannin fractions were further separated on Sephadex LH-20, Diaion HP20SS, Chromatorex ODS, and preparative HPLC to yield six new ellagitannins herein named rubusuaviins A—F (**8**—**13**) along with seven known tannins. The known tannins were identified as pedunculagin (**1**),[6] 1(β)-*O*-galloyl pedunculagin (**2**),[13] strictinin (**3**),[14] sanguiin H-5 (**4**),[7,8] lambertianin A (**5**),[4,15] sanguiin H-6 (**6**)[5—8] and 1-desgalloyl sanguiin H-6 (**7**)[7,8] by direct comparison of their spectral and physical data with those of authentic samples (Fig. 2).

Compound **8** was isolated as a pale brown, amorphous powder and characterized as an ellagitannin by its characteristic colorations with ferric chloride reagent (dark blue) and sodium nitrate–AcOH reagent (brown) on thin-layer chromatography (TLC). The MALDI-TOF-MS showed a $[M+Na]^+$ peak at *m/z* 1127, and the molecular formula $C_{48}H_{32}O_{31}$ was confirmed by elemental analysis. The ^{1}H-NMR spectrum exhibited one two-proton singlet (δ 7.12) and two one-proton singlet (δ 6.34, 6.35) signals attributable

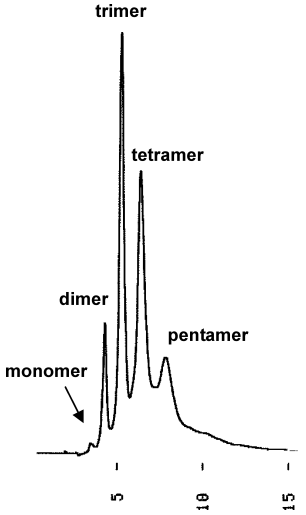

Fig. 1. Normal Phase HPLC of the Fraction 6 Containing Ellagitannin Oligomers

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to a galloyl and an HHDP ester group, respectively. Furthermore, the appearance of a pair of characteristic *meta*-coupled aromatic doublets (δ 7.13, 7.17, each J=2.0 Hz) along with a one-proton singlet (δ 6.72) suggested the presence of a sanguisorboyl group. These signals are similar to those of sanguiin H-2 [1-*O*-galloyl-2,3-(*S*)-HHDP-4,6-(*S*)-sanguisorboyl-α-D-glucose].[5—8] The acyl groups were also confirmed by observation of the aromatic and carboxyl signals in the ^{13}C-NMR spectrum. The appearance of seven aliphatic signals in the range δ 5.91—3.82 with large coupling constants ($J_{1,2}$=8.3 Hz, $J_{2,3}$=$J_{3,4}$=$J_{4,5}$=8.3—10.0 Hz) indicated the presence of a fully acylated β-D-glucopyranose core adopting the ^{4}C$_1$ conformation. The sugar and its absolute configuration were further confirmed by complete acid hydrolysis and subsequent conversion to an arylthiocarbamoyl-thiazolidine derivative by reaction with L-cysteine and *o*-tolyl isothiocyanate.[16] The geminal-coupled glucose H-6 protons resonated at δ 5.58 (dd, J=6.5, 13.5 Hz) and 3.82 (br d, J=13.5 Hz), and a large difference in the chemical shifts of these signals was commonly observed in the ellagitannins with the (*S*)-HHDP or sanguisorboyl groups at the 4,6-positions.[17] In addition, compared with the ^{1}H-NMR signals of 1(β)-*O*-galloyl-pedunculagin (**2**) the H-1, H-3, and H-5 signals were largely shifted to the upper field [$\Delta\delta$ −0.25 (H-1), −0.1 (H-2), −0.51 (H-3), −0.18 (H-4), −0.51 (H-5), 0.29 (H-6), −0.03 (H-6)]. The upfield shifts of these protons located on the α-side of the pyranose ring were caused by the anisotropic effect of the additional galloyl group of the sanguisorboyl group, which indicates the orientation of the sanguisorboyl group.[4] The locations of the acyl group were confirmed based on the HMBC correlations between glucose protons, ester carboxyl carbons, and aromatic protons (Fig. 3). The atropisomerism of the chiral HHDP and sanguisorboyl groups was determined to be *S* based on the circular dichroism (CD) spectrum, which exhibited a strong positive Cotton effect at 237 nm and a negative one at 264 nm.[18] Moreover, selective hydrolysis of the galloyl group by treatment with tannase yielded desgalloylsanguiin H-2, which was identified by comparison with an authentic sample prepared by similar hydrolysis of sanguiin H-2.[7,8] Consequently, the structure of compound **8** was confirmed to be 1-*O*-galloyl-2,3-*O*-(*S*)-HHDP-4,6-*O*-(*S*)-sanguisorboyl-β-D-glucopyranose and was named rubusuaviin A.

Compound **9** was characterized as a dimeric ellagitannin based on its brown coloration with sodium with nitrate–AcOH reagent and a $[M+Na]^+$ ion peak at m/z 1895 in the MALDI-TOF-MS. In the ^{1}H-NMR spectrum, three two-proton singlet signals (δ 6.85, 6.91, 7.05), five one-proton sin-

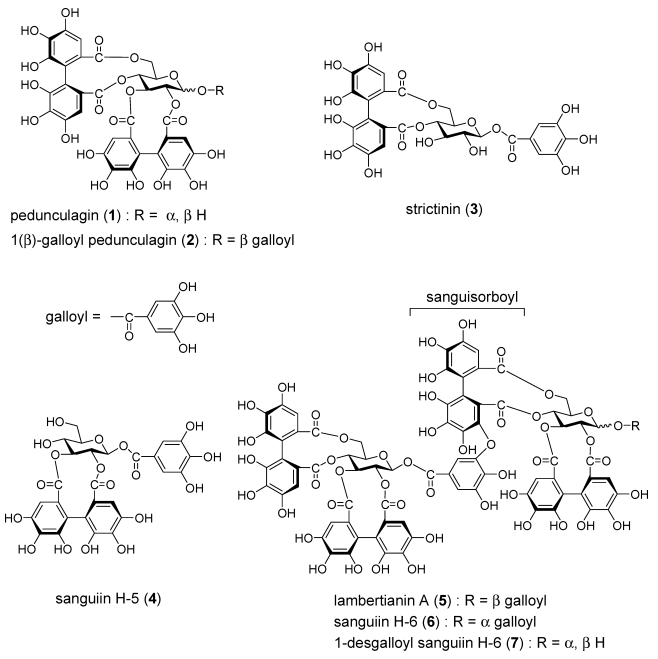

Fig. 2. Structures of Known Ellagitannins

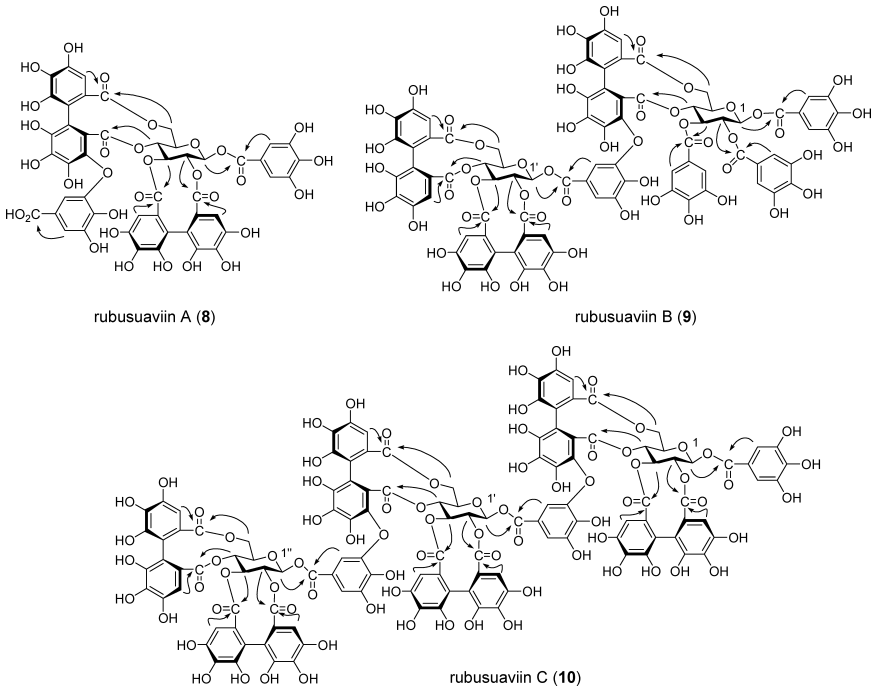

Fig. 3. Structures of **8**—**10** and the Important HMBC Correlations

glet signals (δ 6.33, 6.52, 6.54, 6.65, 6.76), and a pair of *meta*-coupled doublet signals (δ 7.02, 7.05, J=2 Hz) indicated the presence of three galloyl, two HHDP, and one sanguisorboyl groups. The acyl groups were confirmed based on the ^{13}C-NMR signals, and the configuration of the biphenyl bonds of the HHDP and sanguisorboyl groups was shown to be *S* series based on a large negative Cotton effect at 264 nm and a positive one at 237 nm in the CD spectrum.[18] The sugar proton signals were assigned based on ^{1}H–^{1}H COSY spectral analysis. The chemical shifts of anomeric [δ 5.98 (d, J=8.1 Hz) and 6.23 (d, J=8.5 Hz)] and remaining proton (δ 4.01—5.70) signals indicated that all the hydroxyl groups of two hexoses were acylated. In addition, their large coupling constants ($J_{1,2}$, $J_{2,3}$, $J_{3,4}$, $J_{4,5}$=8.0—10.1 Hz) revealed that both sugar moieties were β-glucopyranose with the ^{4}C$_{1}$ conformation. Large chemical shift differences for the glucose H-6 methylene protons [δ 4.01 and 5.70 for H-6, δ 4.03 and 5.30 for H-6′] were similar to those of **2** and indicated that the HHDP and sanguisorboyl ester groups are bridged over the glucose C-2 and C-3 and/or C-4 and C-6 positions.[17] Furthermore, HMBC correlations between pyranose ring protons and ester carbonyl carbons of the respective acyl groups confirmed the location as shown in Fig. 3. Based on the spectral observations, the structure of **9** was determined as shown in Fig. 3 and was named rubusuaviin B.

Compound **10** was isolated as a pale brown, amorphous powder and the MALDI-TOF-MS gave a [M+Na]$^{+}$ ion peak at *m*/*z* 2845, indicating that **10** is an ellagitannin trimer. The ^{1}H-NMR spectrum was similar to that of **9**, showing signals arising from pyrogallol ring and acylated sugar moieties. However, the spectrum exhibited 21 aliphatic proton signals attributable to three β-glucopyranoses, which were confirmed by acid hydrolysis and HPLC analysis after condensation with L-cysteine methyl ester and *o*-tolyl isothiocyanate. The low-field shift of the glucose protons and their large coupling constants indicated that all of the hydroxyl groups were esterified and the pyranose was adapted to a ^{4}C$_{1}$ conformation. The presence of a galloyl ester [δ 7.13 (2H, s)] and two sanguisorboyl groups [δ 7.16, 7.21, 7.19, 7.23 (each 1H, d, J=1.8 Hz), 6.79, 6.81 (each 1H, s)] were deduced from the appearance of their characteristic aromatic signals, and the remaining eight aromatic singlet signals were ascribable to the HHDP protons. The ^{13}C-NMR signals confirmed the presence of these acyl groups (see Experimental). The CD spectrum suggested that the atropisomerism of the biphenyl bonds was *S* series, which was confirmed by methylation and alkaline hydrolysis.[5,7,8]

Complete assignments of the sugar proton and carbon signals were achieved by performing ^{1}H–^{1}H-COSY, HMQC, and HMBC spectral analyses, and the location of the acyl groups was determined based on the HMBC correlations between the sugar proton signals and ester carbonyl carbons (Fig. 3). Compared with the H-3″ (δ 5.44) of the terminal unit, large upfield shifts of the signals due to H-3 (δ 4.91) and H-3′ (δ 4.97) of the glucose-bearing sanguisorboyl esters were observed consistently for **8**, supporting the orientation of the sanguisorboyl esters at the glucose C-4 and C-6 positions. Thus the structure of compound **10** was determined as shown in Fig. 3 and was named rubusuaviin C.

Compound **11** showed an [M+Na]$^{+}$ ion peak at *m*/*z* 2693 in MALDI-TOF-MS, indicating that the molecular weight was 152 mass units less than that of **10**. The ^{1}H-NMR spectrum was also similar to that of **10**. However, signals attributable to galloyl groups were not observed, and complex sugar proton signals suggested the presence of anomeric equilibrium due to the occurrence of a free anomeric hydroxyl group. The spectral observations strongly suggested that **11** is a desgalloyl derivative of **10**, which was confirmed by se-

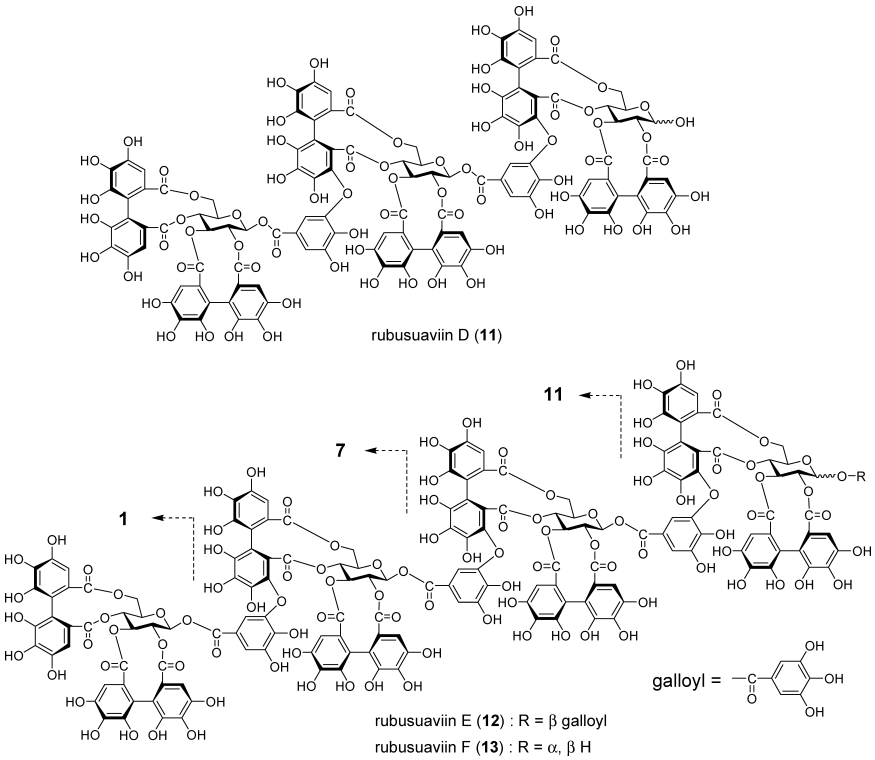

Fig. 4. Structures of **11**—**13**

lective hydrolysis of the galloyl group of **10** with tannase to yield **11**. Therefore the structure of **11** was determined as shown in the Fig. 4 and the compound was named rubusuaviin D.

Compound **12** had a smaller *Rf* value (0.26) on silica gel thin-layer chromatography [dioxane–ethyl formate–formic acid (2 : 10 : 3)] compared with that of **10** (0.45). In addition, the retention time (4.3 min) on the normal-phase HPLC suggested that **12** is an ellagitannin tetramer. The ^{1}H-NMR spectrum, which was similar to that of **10**, showed four sugar anomeric proton signals at 6.01, 6.03, 6.09 and 6.21 (each d, J=8.5 Hz), and remaining complex sugar signals in the range of δ 3.94—5.70 indicated that the sugar moieties were fully acylated. The sugar was identified as D-glucose by acid hydrolysis and subsequent condensation with L-cysteine and *o*-tolyl isothiocyanate. Aromatic proton signals suggested the presence of a galloyl (δ 7.14, 2H, s) and three sanguisorboyl groups [δ 6.78, 6.80, 6.81 (each 1H, s), 7.10, 7.15, 7.16 (each 1H, br s), 7.13, 7.16, 7.20 (each 1H, d, J=2.1 Hz)]. The remaining 10 singlet signals in the range of δ 6.24—6.70 were attributable to the aromatic protons of five HHDP groups. Although the overlapping of the signals hampered their assignment, the ^{13}C-NMR spectrum also supported the presence of these acyl groups. Partial hydrolysis of **12** in hot water yielded pedunculagin (**1**), 1-desgalloyl sanguiin H-6 (**7**), rubusuaviin D (**11**), and compound **13** (Fig. 4). Compound **13** was also derived from **12** by treatment with tannase, indicating that compound **13** is the 1-desgalloyl analogue of **12**. Consequently, compound **12** was determined to have the structure shown in Fig. 4 and was named rubusuaviin E. In addition, the hydrolysate **13** was isolated from the same plant source and named rubusuaviin F.

Our results established the structures of the major ellagitannins of *R. suavissimus*, which comprise monomeric to tetrameric ellagitannins. Although the isolation was unsuccessful, the presence of pentameric ellagitannins was also indicated by normal-phase HPLC of the ellagitannin fractions (Fig. 1). The oligomeric ellagitannins have sanguisorboyl esters and are closely related to those of *S. officinalis*. The important difference between the ellagitannins of these two plants is the configuration at the glucose anomeric positions; that is, the major ellagitannins **8**, **9**, **10**, and **12** of *R. suavissimus* have β-galloyl groups at the C-1 positions. In contrast, the major ellagitannins of *S. officinalis*, 1-*O*-galloyl-2,3,4,6-

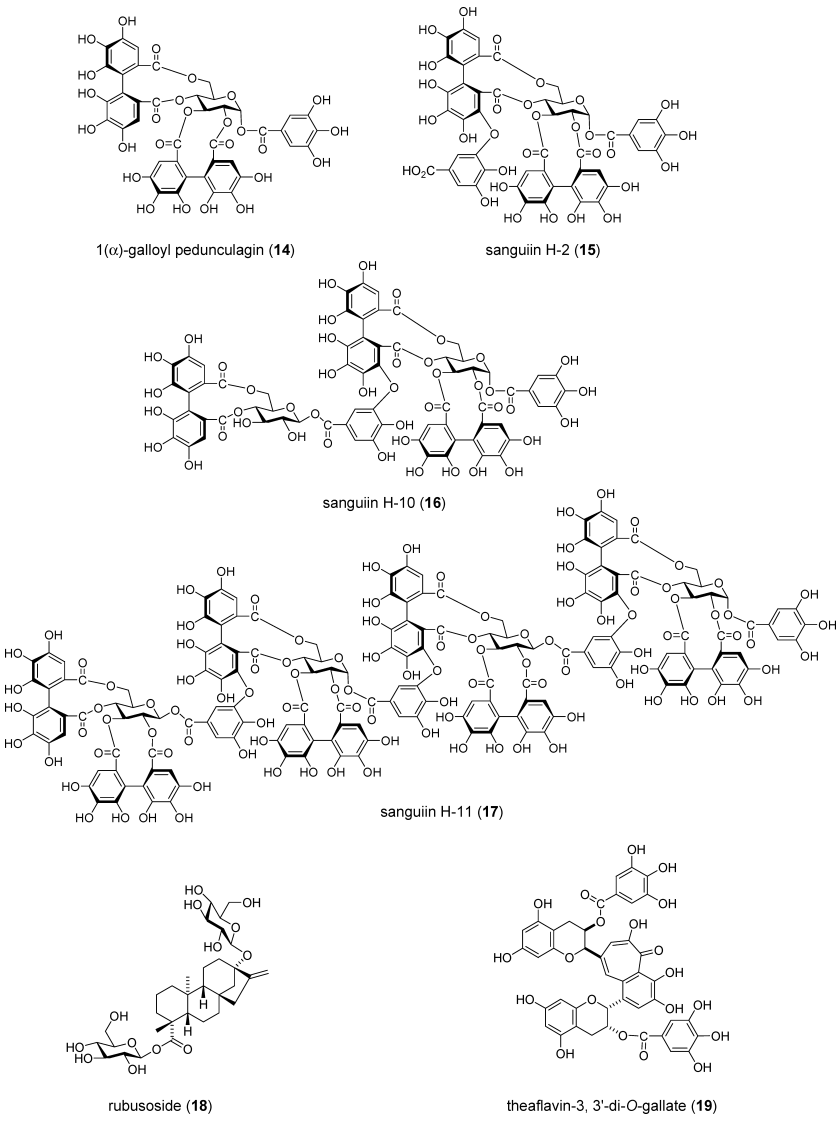

Fig. 5. Structures of **14**—**19**

Table 1. α-Amylase Inhibition Activity

Compound No.		Anomeric configuration	Inhibition (%)
1	Monomer	α,β-OH	14.8±1.5
2	Monomer	β-*O*-Galloyl	56.2±4.4
4	Monomer	β-*O*-Galloyl	52.5±2.5
5	Dimer	β-*O*-Galloyl	56.3±2.7
6	Dimer	α-*O*-Galloyl	36.9±1.8
7	Dimer	α,β-OH	19.0±0.9
8	Monomer	β-*O*-Galloyl	54.2±5.5
9	Dimer	β-*O*-Galloyl	60.6±2.5
10	Trimer	β-*O*-Galloyl	60.8±0.9
11	Trimer	α,β-OH	17.2±1.2
12	Tetramer	β-*O*-Galloyl	52.3±2.7
13	Tetramer	α,β-OH	14.9±1.2
14	Monomer	α-*O*-Galloyl	34.2±2.3
15	Monomer	α-*O*-Galloyl	36.1±4.2
16	Dimer	α-*O*-Galloyl	36.5±5.2
17	Tetramer	α-*O*-Galloyl	23.6±1.2
18			1.3±0.6
19[a)]			83.7±3.6

a) Positive control.

bis-(*S*)-HHDP-α-D-glucose (**14**), sanguiins H-2 (**15**), H-6 (**6**), H-10 (**16**), and H-11 (**17**) have α-galloyl esters (Fig. 5). The difference was reflected in their inhibition of α-amylase activity, which has been linked to a decreased incidence of common diseases caused by diets rich in carbohydrates.[19] The positive control used in this experiment was theaflavin-3,3′-di-*O*-gallate (**19**).[19] As shown in Table 1, ellagitannins with a β-galloyl group showed stronger inhibition compared with those with α-galloyl groups or free hydroxyl groups. The inhibitory effect was not related to molecular size. The results indicate that the α-amylase inhibitory activity of total ellagitannins of *R. suavissimus* was much stronger than that of *S. officinalis* ellagitannins. In addition to the presence of diterpene glycosides as the sweetening principle, the presence of these ellagitannins supports the use of Chinese sweet tea as a complementary medicine to help reduce the occurrence of common diseases, such as diabetes.

Experimental

General Elemental analysis was conducted with a PerkinElmer 2400 II analyzer (PerkinElmer, Waltham, MA, U.S.A.). Infrared (IR) and Ultraviolet (UV) spectra were obtained with JASCO FT/IR-410 and JASCO V-560 spectrophotometers, optical rotations were measured with a Jasco DIP-370 digital polarimeter, and CD spectra were measured on a JASCO J-720W spectropolarimeter. ^{1}H-, ^{13}C-NMR, ^{1}H–^{1}H COSY, NOESY, HSQC, and HMBC spectra were recorded in a mixture of acetone-d_6 and D_2O with a Varian Unity plus 500 spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Coupling constants are expressed in Hz and chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. HMQC, HMBC, and NOESY experiments were performed using standard Varian pulse sequences. Mass spectra (MS) were recorded on a Voyager-DE Pro spectrometer, and 2,5-dihydrohydroxybenzoic acid or α-cyano-4-hydroxycinnamic acid (10 mg/ml in 50% acetone containing 0.05% trifluoroacetic acid) was used as the matrix for MIDLI-TOF-MS measurements. Column chromatography was conducted with Sepabeads SP825, Diaion HP20SS (Mitsubishi Chemical Co.), Chromatorex ODS (Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co.) columns. Thin-layer chromatography (TLC) was performed on 0.2-mm-thick precoated Kieselgel 60 F_{254} plates (Merck) with benzene–ethyl formate–formic acid (1 : 7 : 1, 1 : 5 : 2 v/v), dioxane–ethyl formate–formic acid (2 : 10 : 3, v/v), or cellulose F_{254} (Merck) with 2% AcOH. Spots were detected by UV illumination, sprayed with 2% ethanolic $FeCl_3$ or 10% sulfuric acid reagent, and heated. Analytical reverse-phase HPLC was performed on a Cosmosil 5C_{18}-AR II column (Nacalai Tesque Inc.; 4.6 mm i.d.×250 mm) with gradient elution of 4—30% (39 min) and 30—75% (15 min) CH_3CN in 50 mM H_3PO_4 (flow rate 0.8 ml/min; detection with a JASCO photodiode array detector MD-910). Sanguiins H-2, H-6, and H-11 were used as the standards for monomeric, dimeric, and tetrameric ellagitannins, respectively.[7,8] Preparative HPLC was performed on a Cosmosil 5C_{18}-AR-II column (Nacalai Tesque Inc.; 10 mm i.d.×250 mm) with 20—70% CH_3CN in 0.5% TFA (a linear gradient elution). Tannase extracted from *Aspergillus* sp. was kindly provided by Sankyo Co., Ltd.

Plant Material *R. suavissimus* was collected in Gui-Lin, Guang-Xi province, China. A voucher specimen was deposited in the Kunming Institute of Botany herbarium.

Extraction and Separation Fresh leaves of *R. suavissimus* were steamed for 1—2 min after harvesting to inactivate enzymes that may degrade the chemical constituents, after which the leaves were dried at room temperature. The dried leaves (7 kg) were homogenized with 70% aqueous acetone. After filtration, the filtrate was concentrated *in vacuo* and dried with a spray dryer to yield an extract powder (1.2 kg). The extract was dissolved in H_2O and subjected to Sepabeads SP825 (10 cm i.d.×65 cm) column chromatography. After washing the column with water to elute out sugars and inorganic substances, the remaining compounds were eluted out with water containing increasing proportions of MeOH (0—100%, 10% stepwise elution, each 2 l) to give fractions mainly containing tannins (449 g), flavonoid glycosides (228.7 g), and diterpene glycosides (300.2 g). The tannin fraction was applied to a Sephadex LH-20 column (10 cm i.d.×36 cm) with aqueous MeOH (30, 60, 90, 100%; stepwise elution, each 2 l) and then with H_2O–acetone–MeOH (1 : 1 : 8, 1 : 1 : 3, 2 : 2 : 1, 1 : 1 : 0; stepwise elution, each 1 l) to give seven fractions: frs. 1 (32 g), 2 (80 g), 3 (20 g), 4 (80 g), 5 (105 g), 6 (82 g), and 7 (15 g). Ellagitannins, which showed a brown coloration with sodium nitrate–AcOH reagent, were contained mainly in fr. 4 to fr. 7. Fr. 4 was applied to a Diaion HP20SS (5 cm i.d.×65 cm) column with H_2O–MeOH (10—40%, 10% stepwise elution, each 500 ml) to give a fraction containing ellagitannins (30.3 g), and the fraction was further separated on Toyopearl HW-40C column chromatography (5 cm i.d.×65 cm, 40—100% MeOH and then 50% acetone–H_2O) to yield **1** (4.1 g), **2** (467 mg), **3** (49 mg), and **4** (944 mg). Fr. 5 was fractionated into three fractions on a Diaion HP20SS column (5 cm i.d.×65 cm) with H_2O–MeOH (10—40%). Frs. 5-2 (14.2 g) and 5-3 (12.2 g) were subjected separately to Toyopearl HW-40C column chromatography (5 cm i.d.×65 cm; MeOH–H_2O–acetone, 1 : 0 : 0, 18 : 1 : 1, 8 : 1 : 1, 14 : 3 : 3, 3 : 1 : 1, 0 : 1 : 1, each 500 ml, stepwise elution) and Chromatorex ODS (3.0 cm×50 cm, 0—40% MeOH, 5% stepwise elution) to give **7** (73 mg) and **8** (352 mg). Fr. 6 was applied to a Diaion HP20SS column (5 cm i.d.×65 cm) with H_2O-MeOH (10—50%, 10% stepwise elution) and yielded three fractions. Frs. 6-2 (12.1 g) and 6-3 (6.6 g) were subjected separately to Sephadex LH-20 column chromatography (4 cm i.d.×36 cm; MeOH–H_2O–acetone, 1 : 0 : 0, 18 : 1 : 1, 8 : 1 : 1, 14 : 3 : 3, 3 : 1 : 1, 0 : 1 : 1, each 500 ml, stepwise elution) and Chromatorex ODS (3.0 cm i.d.×30 cm, 0—40% MeOH, 5% stepwise elution) to yield **2** (330 mg), **5** (4.24 g), **6** (221 mg), and **9** (340 mg). Fr. 7 was separated by Toyopearl HW-40C column chromatography (4 cm i.d.×25 cm; MeOH–H_2O–acetone, 1 : 0 : 0, 18 : 1 : 1, 8 : 1 : 1, 14 : 3 : 3, 3 : 1 : 1, 0 : 1 : 1, each 500 ml, stepwise elution) and gave three fractions containing oligomeric ellagitannins: frs. 7-1 (4.8 g), 7-2 (1.6 g), and 7-3 (3.0 g). Fr. 7-1 was applied to a Chromatorex ODS column (3.0 cm i.d.×30 cm, 0—40% MeOH, 5% stepwise elution) to yield **10** (809 mg) and **11** (804 mg). Fr. 7-2 was also purified by Chromatorex ODS column chromatography in a similar manner to give another batch of **10** (70 mg) and **5** (132 mg). Fr. 7-3 was fractionated into four fractions on Chromatorex ODS column chromatography and further purified using preparative HPLC to yield **12** (90 mg) and **13** (60 mg).

Normal-Phase HPLC of Tannin Fractions and Ellagitannins Tannin fractions and isolated ellagitannins were analyzed using LiChroCART 250-4.0 Superspher Si 60 (Kanto Chemical Co., Inc.; 250×4.6 mm i.d.) with *n*-hexane–MeOH–tetrahydrofuran–HCO_2H (100 : 30 : 10 : 1) containing oxalic acid (500 mg/l), 1-*O*-galloyl pedunculagin (MW 936): t_R 3.6 min, sanguiin H-6 (MW 1870): t_R 4.3 min, and sanguiin H-11 (MW 3738): t_R 6.7 min. A linear correlation between the molecular weight and $\log t_R$ value was observed ($MW = 10371 \times \log t_R - 48085$, $R^2 = 0.99$). Fr. 5 showed three peaks at 3.4 min (estimated MW 708), 3.6 min (estimated MW 964), and 4.2 min (estimated MW 1660). Fr. 6 showed two peaks at 4.3 min (estimated MW 964) and 5.1 min (estimated MW 2534). Fr. 6 showed three peaks at 5.2 min (estimated MW 2622), 6.3 min (estimated MW 3486), and 7.8 min (estimated MW 4448).

Rubusuaviin A (8) Pale brown, amorphous powder; $[\alpha]_D$ −7.9° (c=1.0, MeOH); MALDI-TOF-MS m/z: 1127 $[M+Na]^+$; *Anal.* Calcd for

$C_{48}H_{32}O_{31}\cdot 6H_2O$: C, 47.54; H, 3.66. Found: C, 47.56; H, 3.64; IR ν_{max} cm^{-1}: 3402, 1727, 1612, 1509, 1445, 1315, 1192; UV λ_{max} (EtOH) nm (log ε): 260 (4.76), 222 (5.02); CD (MeOH) [θ] (nm): $+2.4\times10^5$ (237), -9.3×10^4 (264), $+6.9\times10^2$ (285), -3.0×10^4 (308); ^{1}H-NMR: δ 3.82 (1H, d, J=13.5 Hz, H-6), 3.94 (1H, dd, J=6.5, 9.9 Hz, H-5), 4.88 (1H, br t, J=10.0 Hz, H-3), 4.93 (1H, t, J=9.9 Hz, H-4), 5.05 (1H, br t, J=8.8 Hz, H-2), 5.58 (1H, dd, J=6.5, 13.5 Hz, H-6), 5.91 (1H, d, J=8.3 Hz, H-1), 6.34, 6,35 (each 1H, HHDP-H-3, 3′), 6.72 [1H, s, sanguisorboyl (sang)-H-3], 7.12 (2H, s, galloyl-2, 6), 7.13 (1H, d, J=2.0 Hz, sang-H-2″), 7.17 (1H, d, J=2.0 Hz, sang-H-6″); ^{13}C-NMR: δ 62.7 (glc-6), 69.4 (glc-4), 73.9 (glc-5), 75.6 (glc-2), 76.8 (glc-3), 92.1 (glc-1), 107.1 (HHDP-3), 107.9 (HHDP-3′), 108.0 (sang-3), 110.0 (galloyl-2, 6), 110.5 (sang-6″), 111.9 (sang-2″), 114.4, 114.9 (HHDP-1,1′), 115.1, 115.3 (sang-1,1′), 119.7 (galloyl-1), 121.0, 121.1 (sang-2′,2″), 125.8, 126.0, 126.3 (HHDP-2,2′, sang-2), 135.4 (sang-3′), 136.1, 136.3, 136.4 (HHDP-5,5′, sang-5), 137.6 (sang-5′), 138.5 (sang-4′), 139.8, 139.9 (galloyl-4, sang-4″), 142.1 (sang-6′), 144.0, 144.2 (HHDP-6 or 6′, sang-6), 144.8, 144.9, 144.9 (HHDP-4,4′,6 or 6′), 145.4 (sang-4), 145.9 (sang-5″), 146.1 (galloyl-3,5), 147.9 (sang-3″), 164.9 (galloyl-7), 165.8 (sang-7′), 167.8 (sang-7), 167.9 (sang-7″), 168.1 (HHDP-7′), 168.5 (HHDP-7).

Tannase hydrolysis of 8 Compound **8** (10 mg) was treated with tannase (5 mg) in H_2O (2 ml) at room temperature for 8 h. The reaction mixture was applied directly to a Sephadex LH-20 column with aqueous MeOH to give gallic acid and hydrolysate (3 mg), which was identified as desgalloylsanguiin H-2 by ^{1}H-NMR spectral comparison.

Rubusuaviin B (9) Pale brown, amorphous powder; $[\alpha]_D$ −7.3° (c=1.0, MeOH); MALDI-TOF-MS m/z: 1895 $(M+Na)^+$; *Anal.* Calcd for $C_{82}H_{56}O_{52}\cdot 8.5H_2O$: C, 48.60; H, 3.60. Found: C, 48.60; H, 3.63; IR ν_{max} cm^{-1}: 3520, 1730, 1614, 1516, 1505, 1455; UV λ_{max} (EtOH) nm (log ε): 267 (5.15), 218 (5.32); CD (MeOH) [θ] (nm): $+3.2\times10^5$ (240), -1.7×10^5 (264), $+1.2\times10^4$ (287), -4.2×10^4 (311); ^{1}H-NMR: δ 4.01 (1H, d, J=13.1 Hz, H-6), 4.03 (1H, d, J=12.8 Hz, H-6′), 4.23 (1H, dd, J=6.4, 9.8 Hz, H-5), 4.47 (1H, br dd, J=6.4, 9.9 Hz, H-5′), 5.13 (1H, t, J=10.1 Hz, H-4′), 5.30 (1H, dd, J=6.4, 13.2 Hz, H-6′), 5.14 (1H, t, J=9.8 Hz, H-4), 5.23 (1H, br t, J=8.8 Hz, H-2′), 5.41 (1H, dd, J=8.8, 10.1 Hz, H-3′), 5.44 (1H, m, H-3), 5.46 (1H, m, H-2), 5.70 (1H, dd, J=6.4, 13.1 Hz, H-6), 5.98 (1H, d, J=8.0 Hz, H-1), 6.23 (1H, d, J=8.2 Hz, H-1′), 6.33 [1H, s, HHDP(glc-3′)-H], 6.52 [1H, s, HHDP(glc-2′)-H], 6.54, 6.65, 6.76 [each 1H, s, HHDP(glc-4′)-H, HHDP(glc-6′)-H, sang-H-3], 6.85 [2H, br s, galloyl(glc-2)-H], 6.91 [2H, s, galloyl(glc-3)-H], 7.02 (1H, br s, sang-H-2″), 7.05 [2H, s, galloyl(glc-1)-H] 7.24 [1H, br s, sang-H-6″]; ^{13}C-NMR: δ 63.0 (2C) (glc-6, 6′), 69.1 (glc-4′), 70.3 (glc-4), 72.1 (glc-2), 72.9 (glc-3), 73.3 (glc-5′), 73.4 (glc-5′), 75.9 (glc-2′), 77.3 (glc-3′), 92.2 (glc-1′), 93.4 (glc-1), 107.1 [HHDP(glc-3′)-3′], 115.1 [HHDP(glc-2′)-3], 107.4, 108.0, 108.1 [HHDP(glc-4)-3, HHDP(glc-6)-3′, sang-3], 109.9 (2C) [galloyl(glc-3)-2,6], 110.1 (4C) [galloyl(glc-1,2)-2,6], 110.3, 112.5 (sang-2″,6″), 114.4, 115.1, 115.2, 115.4, 115.7, 116.0 (HHDP-1,1′, sang-1,1′), 118.9, 119.2, 119.7, 120.0, 120.4 (galloyl-1, sang-2′,2″), 125.4, 125.6, 125.7, 126.0, 126.1 (HHDP-2,2′, sang-2), 135.5 (sang-3′), 136.1, 136.3, 136.5 (2C), 136.6 (HHDP-5,5′, sang-5), 138.1 (sang-5′), 138.1 (sang-4′), 138.9 [galloyl(glc-2)-4], 139.4 [galloyl(glc-3)-4], 139.9 [galloyl(glc-1)-4], 141.3 (sang-4″), 141.9 (sang-6′), 144.27, 144.32, 144.4 (3C), 144.7, 144.9, 145.0, 135.2, 145.4 (2C), 145.8 (2C), 146.0 (4C) (galloyl-3,5, HHDP-4,4′,6,6′, sang-4,6,5″), 148.1 (sang-3″), 165.0 (sang-7″), 165.3 [galloyl(glc-1)-7], 165.8 [galloyl(glc-2)-7], 166.0 [galloyl(glc-3)-7], 166.1 (ang-7′), 168.04, 168.07, 168.13 [HHDP(glc-4′,6′)-7,7′, sang-7], 168.5 [HHDP(glc-2′)-7], 169.4 [HHDP(glc-3)-7′].

Rubusuaviin C (10) Pale brown, amorphous powder; $[\alpha]_D$ −7.1° (c=1.0 , MeOH); MALDI-TOF-MS m/z: 2827 $[M+Na]^+$; *Anal.* Calcd for $C_{123}H_{80}O_{78}\cdot 13.5H_2O$: C, 48.45; H, 3.54. Found: C, 48.43; H, 3.42; IR ν_{max} cm^{-1}: 3500, 1746, 1614, 1516, 1506, 1455; UV λ_{max} (EtOH) nm (log ε): 265 (5.07); 220 (5.38); CD (MeOH) [θ] (nm): $+6.5\times10^5$ (239), -2.3×10^5 (264), $+1.0\times10^3$ (285), -8.4×10^4 (310); ^{1}H-NMR: δ 3.93 (1H, d, J=12.6 Hz, H-6″), 4.04 (1H, d, J=13.5 Hz, H-6), 4.07 (1H, d, J=13.5 Hz, H-6′), 4.12 (2H, m, H-5, H-5′), 4.47 (1H, dd, J=6.6, 9.2 Hz, H-5″), 4.91 (1H, t, J=9.7 Hz, H-3), 4.97 (2H, m, H-3′, H-4′), 5.02 (1H, t, J=9.8 Hz, H-4), 5.11 (1H, t, J=8.7 Hz, H-2), 5.15 (1H, t, J=10.1 Hz, H-4″), 5.16 (1H, br t, J=8.9 Hz, H-2′), 5.19 (1H, t, J=8.9 Hz, H-2″), 5.35 (1H, dd, J=6.5, 13.3 Hz, H-6″), 5.44 (1H, dd, J=9.1, 10.1 Hz, H-3″), 5.72 (1H, dd, J=6.2, 13.3 Hz, H-6′), 5.75 (1H, dd, J=6.6, 13.3 Hz, H-6), 5.99 (1H, d, J=8.5 Hz, H-1), 6.12 (1H, d, J=8.5 Hz, H-1′), 6.21 (1H, d, J=8.5 Hz, H-1″), 6.29 [1H, s, HHDP(glc-3 or 3′)-H], 6.350, 6.355, 6.360 [each 1H, s, HHDP(glc-3 or 3′)-H, HHDP(glc-2″)-H, HHDP(glc-3″)-H], 6.43 [1H, s, HHDP(glc-6″)-H], 6.54 [1H, s, HHDP(glc-4″)-H], 6.57 [1H, s, HHDP(glc-2)-H], 6.68 [1H, s, HHDP(glc-2′)-H], 6.79 [1H, s, sang(glc-6′)-H-3], 6.81 [1H, s, sang(glc-6)-H-3], 7.13 (2H, s, galloyl-H), 7.16, 7.21 [each 1H, d, J=1.8 Hz, sang(glc-1″)-H-2″,6″], 7.19, 7.23 [each 1H, d, J=1.8 Hz, sang(glc-1′)-H-2″, 6″]; ^{13}C-NMR: δ 63.1 (3C) (glc-6, 6′, 6″), 69.2 (glc-4″), 69.3 (glc-4), 69.4 (glc-4′), 73.4 (glc-5″), 73.8 (glc-5), 73.9 (glc-5′), 75.6 (glc-2), 75.8 (glc-2′), 76.0 (glc-2″), 77.0 (glc-3), 77.1 (glc-3′), 77.3 (glc-3″), 92.2 (2C) (glc-1, 1′), 92.4 (glc-1″), 105.9, 107.1 [HHDP(glc-3 or 3′)-3′], 107.2 [HHDP(glc-6″)-3′], 107.4 [HHDP(glc-2)-3], 107.6 [HHDP(glc-4″)-3], 108.0 [HHDP(glc-2″)-3], 108.1 [HHDP(glc-2″)-3], 108.3 [sang(glc-6′)-3], 108.4 [HHDP(glc-3)-3′], 108.7 [sang(glc-6)-3], 110.3(2C) (galloyl-2, 6), 111.2, 112.3, 112.1, 112.6 (sang-2″,6″), 114.3 (2C), 114.4, 114.7, 114.9 (3C), 115.1 (2C), 115.2, 115.8, 115.9 (HHDP-1,1′, sang-1,1′), 118.9, 119.3, 121.1 (2C) (sang-2′,2″), 119.8 (galloyl-1), 125.9 (2C), 126.1, 126.2 (2C), 126.4, 126.5 (2C), 127.0 (2C) (HHDP-2,2′, sang-2), 135.8 (3C), 136.2 (3C), 136.4 (3C), 136.5 (2C), 136.6 (HHDP-5,5′, sang-5,3′), 137.7, 137.9, 138.3 (2C) (sang-4′,5′), 139.9 (galloyl-4), 141.5, 141.7, 142.0, 142.1 (sang-6′,4″), 144.0, 144.1, 144.2, 144.3, 144.5, 144.8, 145.0, 145.3 (HHDP-4,4′,6,6′, sang-4,6), 146.1 (4C) (galloyl-3,5, sang-5″), 148.0, 148.2 (sang-3″), 165.1 (galloyl-7), 165.2 [sang(glc-1″)-7″], 165.3 [sang(glc-1′)-7″], 165.8 [sang(glc-4)-7′], 165.9 [sang(glc-4′)-7′], 167.5 [sang(glc-6′)-7], 167.6 [sang(glc-6)-7], 167.7 [HHDP(glc-4″)-7], 167.9 (2C) [HHDP(glc-2′)-7, HHDP(glc-6″)-7′], 168.0, 168.1 [HHDP(glc-3)-7′, HHDP(glc-3′)-7′], 168.39 [HHDP(glc-2″)-7], 168.44 [HHDP(glc-2)-7], 169.2 [HHDP(glc-3″)-7′].

Methylation and Methanolysis of 10 A mixture of **10** (50 mg), dimethyl sulfate (0.5 ml), and potassium carbonate (200 mg) in dry acetone (30 ml) was heated under reflux for 2 h. After removal of the inorganic salts by filtration, the filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography with toluene–acetone (95 : 5) to wash out the excess dimethyl sulfate, and further elution of the column with toluene–acetone 9 : 1 yielded the crude methyl ether of **10**. The product was dissolved in 5% aqueous NaOH–MeOH (1 : 2, v/v) and heated at 80 °C for 1 h. After removal of MeOH by evaporation, the aqueous solution was acidified with 2 N HCl and partitioned with Et_2O. The Et_2O layer was dried with Na_2SO_4 and mixed with an Et_2O solution of diazomethane at 4 °C for 10 h. After evaporation, the residue was separated on silica gel chromatography with toluene–acetone (97 : 3—95 : 5) to give methyl 3,4,5-trimethoxybenzoate (1.2 mg), dimethyl (*S*)-4,4′,5,5′,6,6′-hexamethoxydiphenoate (5.8 mg), $[\alpha]_D$ −24.0° (c=0.3, $CHCl_3$), and trimethyl (*S*)-octamethylsanguisorbate (3.8 mg) $[\alpha]_D$ −41.0° (c=0.3, $CHCl_3$).

Rubusuaviin D (11) Pale brown, amorphous powder; $[\alpha]_D$ −13.3° (c=1.0, MeOH); MALDI-TOF-MS m/z: 2675 $(M+Na)^+$; *Anal.* Calcd for $C_{116}H_{76}O_{74}\cdot 15H_2O$: C, 47.65; H, 3.67. Found: C, 47.61; H, 3.67; UV λ_{max} (EtOH) nm (log ε): 265 (5.05); 218 (5.35); CD (MeOH) [θ] (nm): $+9.3\times10^5$ (238), -2.7×10^5 (264), $+2.6\times10^4$ (286), -1.1×10^5 (309); ^{1}H-NMR: δ 3.74—3.98 (m, H-6, H-6′, H-6″), 4.18, 4.38, 4.44 (each m, H-5, H-5′, H-5″), 4.67 (t, J=9.7 Hz, β-H-3), 4.72 (t, J=8.8 Hz, β-H-2), 4.87 (d, J=8.3 Hz, β-H-1), 4.82—4.97 (m, H-2, H-3, H-3′, H-4, H-4′), 5.09—5.19 (m, H-2′, H-2″, H-4″), 5.31 (dd, J=6.4, 13.2 Hz, α- and β-H-6″), 5.36 (d, J=3.0 Hz, α-H-1), 5.43 (t, J=10 Hz, α- and β-H-3″), 5.62—5.77 (m, H-6, H-6′), 6.04, 6.09, 6.18, 6.20 (each, d, J=8.5 Hz, H-1′, H-1″), 6.27, 6.32, 6.38, 6.39, 6.46, 6.49, 6.54, 6.67, 6.68 (each s, HHDP-H), 6.79, 6.86, 6.78, 6.84 (each s, sanguisorboyl-H), 7.15, 7.19 (m sanguisorboyl-H); ^{13}C-NMR: δ 62.9, 63.0, 63.4, 63.5 (glc-6), 67.6, 69.1, 69.4, 69.7, 70.1, 72.8, 73.3, 73.4, 73.8, 73.9, 75.3, 75.5, 75.6, 75.7, 75.9, 76.9, 77.1, 77.3, 77.9 (glc-2,3,4,5), 91.4 (α-glc-1), 92.1, 92.2, 92.3 (glc-1′, glc-1″), 95.2 (β-glc-1), 107.2—108.5 (HHDP-3,3′, sang-3), 111.0—112.4 (sang-2″,6″), 114.4—116.0 (HHDP-1,1′, sang-1,1′), 118.6, 119.1, 120.9, 121.0, 121.1, 121.2 (sang-2′,2″), 125.7—126.7 (HHDP-2,2′, sang-2), 135.4—136.6 (HHDP-5,5′, sang-5,3′), 137.8, 138.3 (sang-4′,5′), 141.4—142.0 (sang-6′,4″), 143.8—145.5 (HHDP-4,4′,6,6′, sang-4,6), 146.0, 146.1 (sang-5″), 148.1, 148.2 (sang-3″), 165.2, 165.4, 165.5, 166.1, 166.1, 167.8, 167.9, 168,1, 168.21, 168.24, 168.3, 168.5, 168.51, 168.53, 168.78, 168.9, 169.3 (COO).

Rubusuaviin E (12) Pale brown, amorphous powder; $[\alpha]_D$ −11.7° (c=1.0, MeOH), MALDI-TOF-MS m/z: 3761 $[M+Na]^+$; *Anal.* Calcd for $C_{164}H_{106}O_{104}\cdot 9H_2O$: C, 50.47; H, 2.74. Found: C, 50.45; H, 2.77; IR ν_{max} cm^{-1}: 3413, 1740, 1617, 1515, 1448, 1322, 1314; UV λ_{max} (EtOH) nm (log ε): 260 (5.43), 220 (5.78); CD (MeOH) [θ] (nm): $+1.1\times10^6$ (239), -4.4×10^5 (264), $+1.1\times10^3$ (285), -1.6×10^5 (308); ^{1}H-NMR: δ 3.94 (1H, d, J=12.8 Hz, H-6‴), 3.99, 4.00, 4.07 (each 1H, d, J=13.3 Hz, H-6, H-6′, H-6″), 4.08—4.15 (3H, m, H-5, H-5′, H-5″), 4.45 (1H, dd, J=6.4, 10.2 Hz, H-5‴), 4.90—5.14 (9H, m, H-2, H-2′, H-2″, H-3, H-3′, H-3″, H-4, H-4′, H-4″), 5.13 (1H, t, J=10.2 Hz, H-4‴), 5.18 (1H, t, J=8.8 Hz, H-2‴), 5.34 (1H, dd, J=6.4, 13.5 Hz, H-6‴), 5.42 (1H, t, J=10.3 Hz, H-3‴), 5.59, 5.65, 5.70 (each 1H, dd, J=6.5, 13.5 Hz, H-6, H-6′, H-6″), 6.01, 6.03, 6.09 (each 1H, d,

J=8.5 Hz, H-1, H-1′, H-1″), 6.21 (1H, d, J=8.5 Hz, H-1‴), 6.24, 6.26, 6.33, 6.34, 6.36, 6.41, 6.42, 6.52, 6.54, 6.70 (each 1H, s, HHDP-H), 6.78, 6.80, 6.81 (each 1H, s, sang-H-3), 7.10, 7.15, 7.16 (each 1H, br s, sang-H-2″ or H-6″), 7.13, 7.16, 7.20 (each 1H, d, J=2.1 Hz, sang-H-2″ or 6″), 7.14 (2H, s, galloyl-H); ^{13}C-NMR: δ 62.3(2C), 63.1(2C) (glc-6,6′,6″,6‴), 69.1 (glc-4‴), 69.3, 69.4 (2C) (glc-4,4′,4″), 73.4 (glc-5‴), 73.8, 73.9 (2C) (glc-5,5′,5″), 75.5 (2C), 75.7 (glc-2,2′,2″), 76.0 (glc-2‴), 76.9 (3C) (glc-3,3′,3″), 77.3 (glc-3‴), 92.2 (3C) (glc-1,1′,1″), 92.4 (glc-1‴), 107.2—109.5 (HHDP-3,3′, sang-3), 110.3 (galloyl-2,6), 110.5—112.3 (sang-2″,6″), 114.3—116.5 (HHDP-1,1′, sang-1,1′), 119.7 (galloyl-1), 118.9, 119.1, 119.2, 120.8 (2C), 121.1 (sang-2′,2″), 125.9—126.4 (HHDP-2,2′, sang-2), 135.5—136.5 (HHDP-5,5′, sang-5,3′), 137.5—138.3 (2C) (sang-4′,5′), 139.8 (galloyl-4), 141.4—142.1 (sang-6′,4″), 144.0—145.3 (HHDP-4,4′,6,6′, sang-4,6), 146.1 (galloyl-3,5, sang-5″), 148.1 (2C), 148.1 (sang-3″), 165.2—169.3 (COO).

Partial Hydrolysis of 12 A solution of **12** (20 mg) in water (5 ml) was heated under reflux for 12 h. The precipitates, which were identified as ellagic acid by TLC and IR comparison with an authentic sample,[7,8] were collected by filtration. The filtrate was analyzed on HPLC [column: Cosmosil 5C_{18}-AR II with gradient elution from 10—20% CH_3CN (45 min) in 50 mM H_3PO_4, flow rate: 0.8 ml/min)] to show the peaks corresponding to gallic acid (8.7 min), **1** (8.0, 11.9 min), **7** (15.1, 19.1 min), **11** (18.5, 23.7 min), **13** (19.0, 24.2 min), and ellagic acid (30.2 min).

Rubusuaviin F (13) Pale brown, amorphous powder; $[\alpha]_D$ −7.8° (c=1.0, MeOH); MALDI-TOF-MS m/z: 3609 $[M+Na]^+$; *Anal.* Calcd for $C_{157}H_{102}O_{100}\cdot 12H_2O$: C, 49.59; H, 3.34. Found: C, 49.61; H, 3.37; IR ν_{max} cm^{-1}: 3427, 1740, 1619, 1510, 1449, 1351, 1227; UV λ_{max} (EtOH) nm (log ε): 260 (5.30), 223 (5.57); CD (MeOH) [θ] (nm): $+1.1\times10^6$ (238), -3.3×10^5 (265), $+1.2\times10^3$ (285), -1.3×10^5 (309); ^{1}H-NMR: δ 3.73—3.98 (m, H-6, H-6′, H-6″, H-6‴), 4.02—4.13, 4.27 (m, H-5, H-5′, H-5″), 4.45 (1H, dd, J=6.4, 10.2 Hz, H-5‴), 4.65—5.02 (m), 5.06—5.18 (m), 5.32 (m, H-6‴), 5.34 (d, J=4.0 Hz, α-H-1), 5.41 (t, J=10 Hz, α- and β-H-3‴), 5.55—5.68 (m, H-6, H-6′, 6″), 5.98, 6.01, 6.02, 6.04, 6.07 (each d, J=8.3 Hz, H-1′, H-1″, H-1‴), 6.25, 6.26, 6.31, 6.32, 6.37, 6.38, 6.40, 6.41, 6.47, 6.50, 6.51, 6.52, 6.67, 6.68 (HHDP-H), 6.77, 6.78, 6.785, 6.793, 6.81, 6.84 (sang-H-3), 7.10—7.19 (m, sanh-H-2″,7″).

Tannase Hydrolysis of Rubusuaviin C (10) and E (12) Rubusuaviins C and D (each 20 mg) were treated separately with tannase (5 mg) in H_2O (5 ml) at room temperature for 4 h. The reaction mixture was applied directly to a Sephadex LH-20 column eluted with aqueous MeOH to give gallic acid and **11** (8 mg) and **13** (6 mg), respectively.

Determination of Sugar Absolute Configuration Compounds **8**, **9**, **10**, and **12** (each 2.0 mg) were dissolved separately in 0.1 ml of 1 M HCl and heated at 100 °C for 24 h. Each mixture was neutralized with Amberlite IRA400 (OH^- form) and filtered through a membrane filter (0.45 μm). The filtrate was concentrated under reduced pressure and dried, and the residue was treated with a solution (0.2 ml) of L-cycteine methyl ester in pyridine (2 mg/ml) at 60 °C for 1 h. A solution (0.2 ml) of *o*-tolylisothiocyanate in pyridine (2 mg/ml) was added to the mixture and heated at 60 °C for 1 h. The resulting solution was analyzed using HPLC [Cosmosil 5C_{18}-AR II column (Nacalai Tesque Inc.; 4.6 mm i.d.×250 mm) with 25% CH_3CN in 50 mM H_3PO_4, flow rate 0.8 ml/min, UV detection at 254 nm]. All samples showed peaks at 17.6 min, which coincided with the t_R value of the tolylthiocarbamoyl-thiazolidine derivative produced from D-glucose. The derivative synthesized from L-glucose was detected at 16.1 min.

α-Amylase Inhibitory Activity Amylase inhibitory activity was assayed according to a published protocol.[19] The enzyme solution was prepared by dissolving human salivary α-amylase (EC 3.2.1.1, type VI, Sigma) in 20 mM phosphate buffer, pH 6.9, containing NaCl 6.7 mM (0.03 mg/ml). A solution of test compound (600 μl of 0.025 mg/ml in the same buffer) was mixed with the enzyme solution (500 μl) in a screw-top plastic tube. The tubes were incubated at 37 °C for 10 min. The reaction was initiated by the addition of 400 μl of starch solution (3 mg/ml in above buffer), and the tubes were incubated at 37 °C for 1 h. Final concentration of the test compound in the incubation mixture was 0.01 mg/ml. An aliquot (400 μl) of the mixture was transferred to a separate tube containing 400 μl of DNS color reagent solution (44 mM 3,5-dinitrosalicylic acid, 10.6 M sodium potassium tartarate in 0.4 M NaOH) and heated at 95 °C for 5 min. After cooling on ice, the solution was diluted by the addition of 4 ml of water and the absorption at 540 nm was measured. The enzyme solution was replaced with phosphate buffer for the blank test, and theaflavin-3,3′-di-*O*-gallate (**19**) was used as a positive control.[19] The adsorption was measured for three replicates of each sample.

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References and Note

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