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Chemical Constituents from the Polar Fraction of *Rubus suavissimus*

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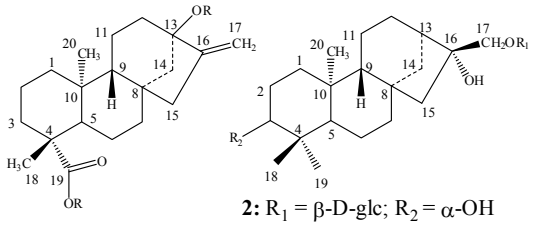
Abstract

Systematic phytochemical study of the *n*-BuOH fraction of the aqueous extract of *Rubus suavissimus* resulted in the isolation of three diterpene glycosides namely rubusoside, suavioside-A and sugeroside; a phenolic glycoside quercetrin; and a lignan glycoside arctiin. The structures of the isolated compounds were characterized on the basis of extensive spectral data (1D and 2D NMR; and MS) and chemical studies which has not been reported earlier. This is the first report of the isolation of quercetrin and arctiin not only from the plant *R. suavissimus* but also from the genus *Rubus*. Also, herewith we are reporting the sweetness recognition threshold and sweetness enhancement effect of rubusoside, the major constituent of *R. suavissimus*.

Keywords: *Rubus suavissimus*, Rosaceae, Diterpene glycosides, Phenolic glycoside, Lignan glycoside, NMR, MS, rubusoside, sweetness recognition threshold, sweetness enhancement effect

Introduction

Rubus suavissimus S. Lee belongs to *Rubus*, a large genus of flowering plants in the rose family, Rosaceae , subfamily Rosoideae. Raspberries, blackberries, and dewberries are common, widely distributed members of this genus. *R. suavissimus* is a perennial shrub grows widely grown in Guang-xi and Guang-dong, China [1]. The leaves of *R. suavissimus* are used to make beverage leaf tea by the local residents because of its intensely sweet flavor. It is generally known as tiancha in Chinese or Chinese sweet tea. Previous phytochemical studies of this plant mainly showed the presence of diterpene and triterpene glycosides as well as phenolic compounds [2-4]. The major constituent of this plant is the sweet diterpenoid glycoside rubusoside with an aglycone moiety belongs to the class of the diterpene, *ent*-13-hydroxykaur-16-en-19-oic acid, known as steviol [5]. As a part of our research to discover natural sweeteners, we have recently reported several ditepene glycosides from *S. rebaudiana* [6-8] and triterpene glycosides from *Siraitia grosvenorii* [9].



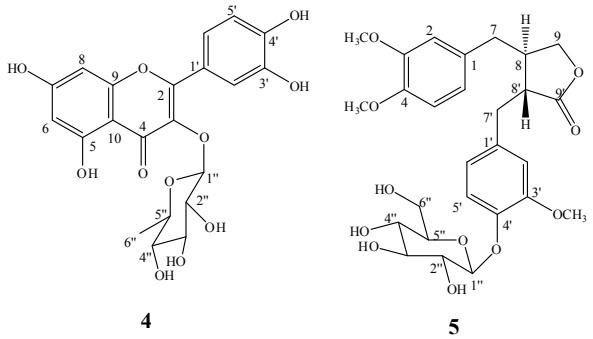
1: R = β-D-glc

2: R_1 = β-D-glc; R_2 = α-OH

3: R_1 = β-D-glc; R_2 = =O

6: R = H

7: R_1 = β-D-glc; R_2 = β-OH



4

5

Figure 1: Structures of **1-5** and other compounds.

In our continuing research to isolate natural compounds from various sweet taste plants collected from all over the World, we have isolated three diterpene glycosides rubusoside (**1**), suavioside-A (**2**) and sugeroside (**3**) as well as the phenolic and lignin glycosides namely quercetrin (**4**) and arctiin (**5**) respectively from the polar fraction of the aqueous extract of the leaves of *R. suavissimus* obtained from Chengdu Biopurify Phytochemicals Limited, China. This paper describes the isolation and structure elucidation of the isolated glycosides **1-5** (Figure 1) on the basis of extensive spectroscopic and chemical studies as well as in comparison of their physical and spectral properties reported from the literature. Also, we are herewith reporting the sweetness recognition threshold (SRT) and sweetness enhancement effect (SEE) of the predominant constituent of the plant, rubusoside (**1**).

Results and Discussion

Compound **1** was isolated as a white powder and its molecular formula has been deduced as $C_{32}H_{50}O_{13}$ on the basis of its HRMS which showed $[M+NH_4]^+$ and $[M+Na]^+$ ions at *m/z* 660.3590 and 665.3136 respectively, and this was supported by the ^{13}C NMR spectral data. The ^1H NMR spectrum of **1** showed the presence of two methyl singlets at δ 1.24 and 1.26, two olefinic protons as singlets at δ 5.03 and 5.56 of an exocyclic double bond, nine methylene and two methine protons between δ 0.79-2.72 characteristic for the *ent*-kaurane diterpenoids isolated earlier from the plants belongs to the genus *Stevia* and *Rubus* [6-8,10,11]. The basic skeleton of kaurane diterpenoids was supported by COSY (H-1/H-2; H-2/H-3; H-5/H-6; H-6/H-7; H-9/H-11;

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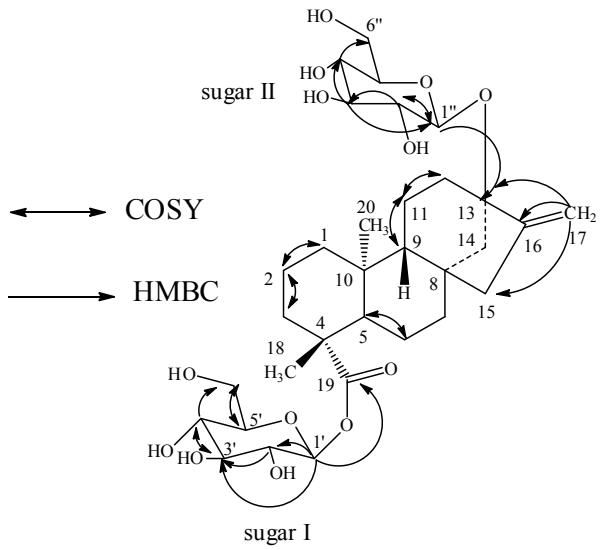

Figure 2: Key COSY and HMBC correlations of **1**.

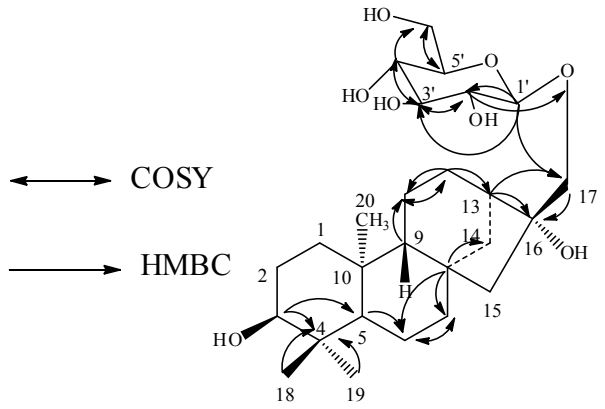

Figure 3: Key COSY and HMBC correlation of **2**.

H-11/H-12) and HMBC (H-1/C-2, C-10; H-3/C-1, C-2, C-4, C-5, C-18, C-19; H-5/C-4, C-6, C-7, C-9, C-10, C-18, C-19, C-20; H-9/C-8, C-10, C-11, C-12, C-14, C-15; H-14/C-8, C-9, C-13, C-15, C-16 and H-17/C-13, C-15, C-16) correlations. The fragment ions observed at *m/z* 481 and 319 in the positive ESI mode MS/MS spectrum of **1** indicating the presence of two hexose sugars in its structure. This was further supported by the ^{1}H NMR spectrum of **1** which showed the presence of two anomeric protons at δ 5.13, and 6.15. Enzymatic hydrolysis of **1** furnished an aglycone which was identified as steviol (**6**) by comparison of ^{1}H NMR [10] spectral data reported in the literature and co-TLC with standard compound. Acid hydrolysis of **1** afforded D-glucose that was identified by preparing the corresponding thiocarbamoyl-thiazolidine carboxylate derivative with L-cysteine methyl ester and *O*-tolyl isothiocyanate, and in comparison of its retention time with the standard sugars as described in the literature comparison [12]. The large coupling constants observed for the two anomeric protons at δ 5.13 (*J*=7.8 Hz), and 6.15 (d, *J*=8.5 Hz) suggested their β-orientation as reported for steviol glycosides [6-8].

The ^{13}C NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations (Table 1 and Table 2). From COSY, and HMBC correlations as shown in Figure 2, **1** was found to have a steviol aglycone moiety having a β-D-glucopyranosyl unit attached C-13 hydroxyl and another β-D-glucopyranosyl moiety in the form of an ester at C-19. This was supported by the HMBC correlations: H-1'/C-19, C-2', C-3'; and H-1"/C-13, C-2", C-3". A close comparison of the NMR spectral data of **1** with the reported literature values for rubusoside confirmed its structure.

The molecular formula of compound **2** was established as $C_{26}H_{44}O_8$ from its HRMS spectral data which showed $[M+NH_4]^+$ and $[M+Na]^+$ ions at *m/z* 502.3327 and 507.2924 respectively. The ^{1}H NMR spectrum of **2** showed the presence of three methyl singlets at δ 0.87, 1.02, and 1.17, eight methylene and two methine protons between δ 0.89-2.43, similar to **1**. The ^{1}H NMR of **2** also showed the presence of signal at δ 4.12 as a triplet like with $W_{1/2}$ = 2.6 Hz and a pair of doublets corresponding to an oxymethylene at δ 3.91 (*J*=10.6 Hz), 4.49 (*J*=10.2 Hz) and an anomeric proton as a doublet at δ 5.05. Acid hydrolysis of **2** afforded D-glucose that was identified by preparing its corresponding thiocarbamoyl-thiazolidine carboxylate derivative as in 1, and the coupling constant observed for the anomeric proton *J*=8.2 Hz suggested the β-orientation of the D-glucosyl unit. The ^{13}C NMR spectrum of **2** showed the presence of nine oxygenated carbons between δ 63.2 and 107.2 of which six were assigned to the β-D-glucopyranosyl unit, leaving the assignment of the other three carbons.

Position	1	2	3
1	0.79 m, 1.68 m	0.89 m, , 1.86 m	1.25 m, 1.98 m
2	1.41 m, 1.92 m	1.34 m, 1.94 m	1.82 dd (13.2, 12.2), 2.54 dd (12.0, 4.8)
3	1.06 m, 2.34 d (12.4)	4.12 t ($W_{1/2}$, 2.60)	-
5	1.32 m	1.57 m	1.83 m
6	1.42 m, 1.72 m	1.42 m, 1.64 m	1.40 m, 1.63 m
7	1.32 m, 1.70 m	1.47 m, 1.66 m	1.45 m, 1.67 m
9	0.94 m	0.98 m	0.97 m
11	1.74 m	1.65 m	1.68 m
12	1.76 m, 1.95 m	1.54 m, 1.70 m	1.51 m, 1.73 m
13	-	2.02 m	1.98 m
14	2.24 m, 2.72 d (12.8)	1.78 m, 2.41 m	1.76 m, 2.43 m
15	2.08 m, 2.52 m	1.35 m, 1.82 m	1.35 m, 1.82 m
17	5.03 s, 5.56 s	3.91 d (10.6), 4.49 d (10.2)	3.95 d (10.4), 4.50 d (10.8)
18	1.26 s	0.87 s	0.93 s
19	-	1.02 s	1.02 s
20	1.24 s	1.17 s	1.10 s
1′	6.15 d (8.5)	5.05 d (8.2)	5.06 d (7.8)
2′	3.96 m	4.04 m	4.02 m
3′	4.08 m	4.46 m	4.45 m
4′	4.33 m	4.25 m	4.23 m
5′	4.21 m	4.63 m	4.64 m
6′	4.03 dd (4.2, 12.4), 4.41 dd (2.4, 9.2)	3.61 dd (4.4, 12.2), 4.27 dd (2.5, 10.6)	3.60 dd (4.4, 12.0), 4.24 dd (2.3, 10.2)
1″	5.13 d (7.8)		
2″	4.19 m		
3″	4.35 m		
4″	4.23 m		
5″	3.38 m		
6″	4.22 dd (3.9, 12.2), 4.62 dd (2.2, 8.0)		

[a]assignments made on the basis of COSY, HSQC and HMBC correlations; [b]Chemical shift values are in δ (ppm); [c]Coupling constants are in Hz.

Table 1: ^{1}H NMR chemical shift values for **1–3** isolated from *Rubus suavissimus* recorded in C_5D_5N [a-c].

Position	1	2	3
1	41.2	34.2	39.6
2	19.9	27.4	34.6
3	38.8	75.6	217.3
4	44.4	38.5	47.5
5	57.8	49.4	54.6
6	22.6	20.8	22.3
7	42.2	42.9	41.6
8	42.9	45.3	44.9
9	54.3	57.2	55.9
10	40.2	40.0	39.0
11	21.1	18.9	19.3
12	37.7	26.9	27.0
13	86.4	47.0	46.7
14	44.9	37.9	37.5
15	48.3	53.8	53.2
16	155.0	81.3	81.2
17	104.9	76.0	75.9
18	28.8	29.8	27.7
19	177.4	22.8	21.5
20	16.1	18.4	18.2
1′	95.4	107.2	107.1
2′	75.9	75.8	76.0
3′	79.5	79.0	79.0
4′	72.8	72.0	72.1
5′	78.5	79.3	79.2
6′	63.5	63.2	63.2
1″	100.2		
2″	74.5		
3″	79.3		
4″	71.5		
5″	79.8		
6″	61.5		

[a]assignments made on the basis of HSQC and HMBC correlations; [b] Chemical shift values are in δ (ppm)

Table 2: ^{13}C NMR chemical shift values for **1–3** isolated from *Rubus suavissimus* recorded in C_5D_5N [a-b].

From HSQC and HMBC spectral data, it was found that the other three oxygenated carbons of **2** as one oxymethylene, one oxymethine and a tertiary hydroxyl resonating at δ 76.0, 75.6 and 81.3 respectively in its ^{13}C NMR spectrum. From the above spectral data and chemical studies, the structure was identified as an *ent*-kaurane diterpenenoid skeleton having a β-D-glucopyranosyl unit and three oxygenated carbons as mentioned above. A search from the literature indicated the presence of two compounds with the above functional groups namely *ent*-kaurane-3α, 16β, 17-triol-17-*O*-β-D-glucoside (suavioside A) and *ent*-kaurane-3β, 16β, 17-triol-17-*O*-β-D-glucoside (iwayoside A, **7**) isolated from *R. suavissimus* [13] and *Artemisia iwayomogi* [14] respectively. The key COSY and HMBC correlations as displayed in Figure 3 supported the basic skeleton of *ent*-kaurane-3, 16, 17-triol-17-*O*-β-D-glucoside. Since the spectral data for suavioside A and iwayoside A were reported in CD_3OD and C_5D_5N respectively and in order to compare the NMR values of **2** with the isolated compounds, its ^{1}H and ^{13}C NMR were also acquired in both the solvents. A close comparison of the NMR spectral data and optical rotation of **2** confirmed the structure as suavioside A.

Compound **3** was also obtained as a white amorphous powder and its molecular formula was inferred as $C_{26}H_{42}O_8$ from its HRMS spectral data that showed $[M+NH_4]^+$ and $[M+Na]^+$ ions at *m/z* 500.3220 and 505.2768 respectively, and this molecular composition was supported by the ^{13}C NMR spectral data. The ^{1}H NMR spectrum of **3** showed the presence three methyl singlets, eight methylene and two methine protons, two protons corresponding to an oxymethylene group and an anomeric proton; identical to **2**. Acid hydrolysis confirmed the sugar and its configuration as D-glucose. A close comparison of the ^{1}H and ^{13}C NMR chemical shift values of **2** and **3** together with the ESI-MS data which has 2 amu difference, suggested the presence of a carbonyl group in **3** at C-3 position in place of an oxymethine proton in **2**. This was further supported by the presence of the peak observed in its ^{13}C NMR spectrum at δ 217.3, and the absence of a triplet like signal for the oxymethine proton at C-3 position and its corresponding carbon in the respective proton and carbon NMR spectral data of **3**. The above spectral and chemical data suggested its structure as **3**, which was reported as sugeroside earlier from *Ilex sugerokii* var. *brevipedunculata* [15] and *R. suavissimus* [13].

Compound **4** was obtained as yellow amorphous powder and its molecular formula was established as $C_{21}H_{20}O_{11}$ from its HRMS spectral data that showed $[M+H]^+$ and $[M+Na]^+$ ions at *m/z* 449.1075 and *m/z* 471.0892 respectively; this was supported by the ^{13}C NMR spectral data.

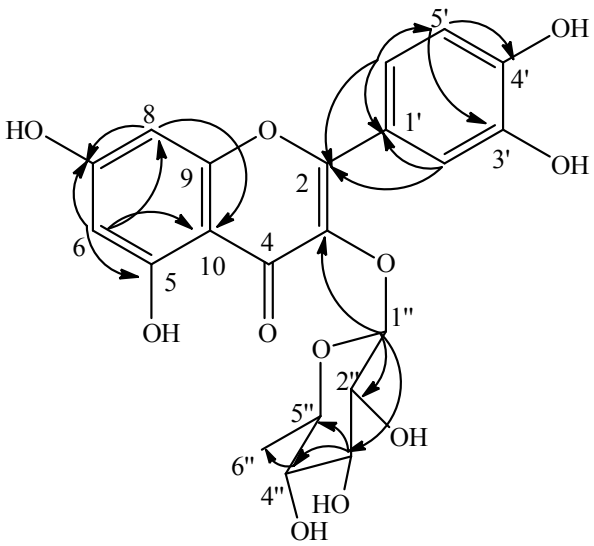

Figure 4: Key HMBC correlation of **4.**

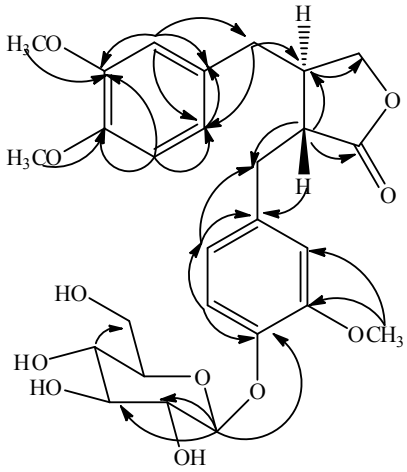

Figure 5: Key HMBC correlations of **5**.

Position	4		5	
	δ_H	δ_C	δ_H	δ_C
1				133.0
2		158.2	6.76 d (1.7)	113.1
3		136.5		150.3
4		179.6		149.1
5		163.5	6.93 d (7.2)	114.7
6	6.65 d (1.6)	95.0	6.72 dd (7.2, 1.9)	121.7
7		166.3	2.83 m	38.4
8	6.72 d (1.8)	100.2	2.52 m	42.0
9		158.7	3.93 dd (7.5, 8.9), 4.20 dd (7.3, 9.2)	71.7
10		105.9		
1′		122.8		132.1
2′	8.03 d (1.7)	117.	7.02 d (1.9)	113.6
3′		147.8		150.1
4′		151.0		147.4
5′	7.31 d (8.1)	116.9	7.58 d (7.5)	116.7
6′	7.73 dd (7.5, 1.8)	122.6	6.88 dd (7.2, 1.6)	122.7
7′			3.07 m	35.0
8′			2.72 m	47.1
9′				179.4
1″	6.29 d (1.5)	104.5	5.69 d (7.2)	102.9
2″	5.08 dd (4.3, 1.6)	72.5	4.20 dd (7.2, 9.4)	75.3
3″	4.65 dd (9.3, 3.3)	72.7	4.33 dd (9.2, 7.8)	79.0
4″	4.33 dd (9.8, 7.2)	73.8	4.35 dd (9.4, 8.2)	71.8
5″	4.41 m	73.0	4.12 ddd (9.4, 1.8, 7.9)	79.3
6″	1.49 d (6.0)	18.1	4.40 dd (2.1, 12.4), 4.55 dd (4.2, 12.2)	62.8
OCH_3			3.75	56.4
OCH_3			3.78	56.5
OCH_3			3.80	56.5

[a]assignments made on the basis of COSY, HSQC and HMBC correlations; [b]Chemical shift values are in δ (ppm); [c]Coupling constants are in Hz

Table 3: ^{1}H and ^{13}C NMR chemical shift values for **4–5** isolated from *Rubus suavissimus* recorded in C_5D_5N[a-c].

The ^{1}H NMR spectrum of **4** showed the presence of three meta coupled aromatic protons at δ 6.65 (*J*=1.6 Hz), 6.72 (*J*=1.8 Hz) and 8.03 (*J*=1.7 Hz); one ortho coupled aromatic proton at δ 7.31 (*J*=8.1 Hz); another ortho and meta coupled aromatic proton as doublet of doublets at δ 7.73 (*J*=7.5, 1.8 Hz); characteristic for the 3-substituted flavone. The ^{1}H NMR of **4** also showed the presence of an anomeric proton as a doublet at δ 6.29 suggesting a sugar residue in its structure which was identified as L-rhamnosyl moiety on the basis of acid hydrolysis and by preparing the corresponding thiocarbamoyl-thiazolidine carboxylate derivative with L-cysteine methyl ester and *O*-tolyl isothiocyanate, and in comparison of its retention time with the standard sugars as described for compounds **1-3**. The presence of L-rhamnosyl moiety was further supported by the presence of the secondary methyl group as a doublet at δ 1.49 (*J*=6.0 Hz) as well as the absence of oxymethylene group at C-5 position of the sugar unit. The anomeric proton had a coupling constant of 1.5 Hz, similar to dulcosides A and B isolated from *S.rebaudiana* [16] confirming the α-orientation L-rhamnosyl moiety. The placement of the L-rhamnosyl moiety was identified at C-3 position on the basis of key HMBC correlations: H-2'/C-2, C-1', C-3' and H-1''/C-2, C-3, C-2'' (Figure 4). Thus, based on the above spectral data the structure of **4** was assigned as quercetin-3-*O*-α-L-rhamnoside (quercetrin) consistent to the reported literature values [17,18].

Compound **5** was obtained as a hard gum and its molecular formula was inferred as $C_{27}H_{34}O_{11}$ from its HRMS spectral data that showed $[M+NH_4]^+$ and $[M+Na]^+$ ions at *m/z* 552.2437 and *m/z* 557.1986 respectively. The ^{1}H NMR spectrum of **5** showed the presence of six aromatic protons between δ 6.72-7.58; three methoxyl groups at δ 3.75, 3.78 and 3.80; two protons of an oxymethylene group at δ 3.93 and 4.20; two methylenes and two methines between δ 2.52-3.07; very similar to the lignan arctigenin. Acid hydrolysis of **5** furnished D-glucose and was identified as having β-orientation from the coupling constant of its anomeric proton appeared at δ 5.69 (*J*=7.2 Hz). From the above spectral data and chemical studies it was evident that the structure of **5** should contain a β-D-glucopyranosyl unit that has been attached to the aglycone moiety of arctigenin. From the HMBC correlations shown in Figure 5, the presence of β-D-glucopyranosyl unit was suggested at C-6' position unambiguously as in arctiin; confirmed its structure completely [19,20].

This is the first report of the isolation of quercetrin and arctiin not only from the plant *R. suavissimus* but also from the genus *Rubus*. Further, the detailed NMR characterizations have not been studied on some of the isolated glycosides and herewith we have assigned the entire proton and carbon values on the basis of COSY, HSQC and HMBC spectral data as well as confirmed the sugars and their configuration by hydrolysis studies. Also, we are reporting the ^{1}H and ^{13}C NMR data for all the isolated five compounds (**1-5**) in C_5D_5N in this article.

Experimental

General

Melting points were measured using a SRS Optimelt MPA 100 instrument and are uncorrected. Optical rotations were recorded using a Rudolph Autopol V at 25 °C and NMR spectra were acquired on a Varian Unity Plus 600 MHz instrument using standard pulse sequences at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. HRMS data was generated with a Thermo LTQ Orbitrap Discovery mass spectrometer in the positive positive ion mode electrospray. Instrument was mass calibrated with a mixture of Ultramark 1621, MRFA [a peptide], and caffeine immediately prior to accurate mass measurements of the samples. Samples were diluted with water:acetonitrile:methanol (1:2:2) and prepared a stock solution of 50 ul concentration for each sample. Each sample (25 ul) was introduced via infusion using the onboard syringe pump at a flow injection rate of 120 ul/min. Low pressure chromatography was performed on a Biotage Flash system using a C-18 cartridge (40+ M, 35-70 µm). TLC was performed on Baker Si-C_{18}F plates and identification of the spots on the TLC plate was carried out by spraying 10% H_2SO_4 in EtOH and heating the plate at about 80°C. Analytical HPLC for sugar analysis was carried out with a Waters 600E multisolvent delivery system using a Phenomenex Luna C_{18} non-chiral (150 x 4.6 mm, 5 µm) column.

Plant material

The commercial sample consisting of the aqueous extract of the leaves of *R. suavissimus* was purchased from Chengdu Biopurify Phytochemicals, China. The plant material was identified by Professor Weiping He, Natural Plant Scientific Institute, Guangdong Ocean University, Guangxi, China and a voucher specimen is deposited at The Coca Cola Company, No. VSPC-3166-68.

Isolation

The aqueous extract of the leaves of *R. suavissimus* (10g) was suspended in 100 ml water and extracted successively with *n*-hexane (3 x 100 ml), CH_2Cl_2 (3 x 100 ml) and *n*-BuOH (2 x 100 ml). The *n*-BuOH

layer was concentrated under vacuum furnished a residue (2.5g) which was purified on a Biotage flash chromatography system using C-18 (100g) column (solvent system: gradient from 20-80 MeOH-water to 100% MeOH at 60 ml/min. detection at UV 210 nm) for 40 min. Fractions 13-20 (0.2g) were combined and further subjected to repeated flash chromatography purification with gradient from 40-80% MeOH-water at 30 ml/min for 30 min afforded suavioside A (**2**, *t*R, 18.6 min, 10.2 mg). Fractions 21-23 (1.4g) were combined and crystallized with MeOH furnished rubusoside (**1**, 1.1g). Purification of the combined fractions 24-27 (0.12g) using the gradient from 40-80% MeOH-water at 30 ml/min for 40 min furnished sugeroside (**3**, *t*R 16.4 min, 11.5 mg). Fractions 46-50 and 52-56 were combined to get residues 0.14 g and 0.11 g respectively, which on repeated purification using the gradient 60-90% MeOH-water at 30 ml/min for 40 min resulted quercetrin (**4**, *t*R 22.4 min, 6.8 mg), and arctiin (**5**, *t*R 19.6 min, 8.4 mg), respectively.

Identification

Rubusoside: White powder, mp 177-179 °C [reported mp 178-181 °C]; $[\alpha]_D^{25}$ -37.20 (*c* 1.0, MeOH) [reported $[\alpha]_D^{18}$ -40.30 (*c* 0.8, MeOH)]; ^{1}H NMR (600 MHz, C_5D_5N, δ ppm) and ^{13}C NMR (150 MHz, C_5D_5N, δ ppm) spectroscopic data see Table 1 and Table 2; HRMS *m/z* $[M+NH_4^+]$ calcd for $C_{32}H_{54}O_{13}N$: 660.3595; found 660.3590 and $[M+Na^+]$ calcd for $C_{32}H_{50}O_{13}Na$: 665.3159; found 665.3136.

Enzymatic hydrolysis of **1**. A solution of 1 (500 µg) was dissolved in 5 ml of 0.1 M sodium acetate buffer, pH 4.5 and crude pectinase from *Aspergillus niger* (100 uL, Sigma-Aldrich, P2736) was added. The mixture was stirred at 50°C for 48 hr. The product precipitated out during the reaction was filtered and then crystallized from methanol (MeOH). The resulting steviol (6, 73 µg) was identical to an authentic sample by co-TLC and ^{1}H NMR [10].

Suavioside A: White powder, $[\alpha]_D^{25}$ +45.62 (*c* 0.15, C_5H_5N) [reported $[\alpha]_D^{25}$ +47.21 (*c* 0.14, C_5H_5N)]; ^{1}H NMR (600 MHz, C_5D_5N, δ ppm) and ^{13}C NMR (150 MHz, C_5D_5N, δ ppm) spectroscopic data see Table 1 and Table 2; HRMS *m/z* HRMS *m/z* $[M+NH_4^+]$ calcd for $C_{26}H_{48}O_8N$: 502.3380; found 502.3327 and $[M+Na^+]$ calcd for $C_{26}H_{44}O_8Na$: 507.2934; found 507.2924.

Sugeroside: White powder, $[\alpha]_D^{25}$ -53.20 (*c* 0.35, C_5H_5N) [reported $[\alpha]_D^{23}$ -55.60 (*c* 0.36, C_5H_5N)]; ^{1}H NMR (600 MHz, C_5D_5N, δ ppm) and ^{13}C NMR (150 MHz, C_5D_5N, δ ppm) spectroscopic data see Table 1 and Table 2; HRMS *m/z* $[M+NH_4^+]$ calcd for $C_{26}H_{46}O_8N$: 500.3223; found 500.3220 $[M+Na^+]$ calcd for $C_{26}H_{42}O_8Na$: 505.2777; found 505.2768.

Determination of the configuration of sugars in **1-3**: Each compound **1-3** (500 µg) was hydrolyzed with 0.5 M HCl (0.5 mL) for 1.5 h. After cooling, the mixture was diluted with 5 ml water, passed through an Amberlite IRA400 column and the eluate was lyophilized. The residue was dissolved in pyridine (0.25 mL) and heated with L-cysteine methyl ester HCl (2.5 mg) at 60°C for 1.5 h, and then *O*-tolyl isothiocyanate (12.5 uL) was added to the mixture and heated at 60°C for an additional 1.5 h. The reaction mixture was analyzed by HPLC: column Phenomenex Luna C18, 150 x 4.6 mm (5 u); 25% acetonitrile-0.2% TFA water, 1 mL/min; UV detection at 250 nm. The sugar was identified as D-glucose in each experiment (*t*R, 12.26 to 12.42 min) [authentic samples, D-glucose (*t*R, 12.35) and L-glucose (*t*R, 11.12 min) [12].

Quercetrin : Yellow powder, mp 179.4-181.2 °C [reported mp 180-182 °C]; ^{1}H NMR (600 MHz, C_5D_5N, δ ppm) and ^{13}C NMR (150 MHz, C_5D_5N, δ ppm) spectroscopic data see Table 3; HRMS *m/z* $[M+H^+]$ calcd for $C_{21}H_{21}O_{11}$: 449.1084; found 449.1075 and $[M+Na^+]$ calcd for $C_{21}H_{20}O_{11}Na$: *m/z* 471.0903; found 471.0892.

Arctiin : Hard gum, $[\alpha]_D^{25}$ -52.60 (*c* 0.003, MeOH) [reported $[\alpha]_D^{23}$ -55.30 (*c* 0.0033, MeOH)]; ^{1}H NMR (600 MHz, C_5D_5N, δ ppm) and ^{13}C NMR (150 MHz, C_5D_5N, δ ppm) spectroscopic data see Table 3; HRMS *m/z* $[M+NH_4^+]$ calcd for $C_{26}H_{48}O_8N$: 552.2445; found 552.2437 and $[M+Na^+]$ calcd for $C_{27}H_{34}O_{11}Na$: *m/z* 557.1999; found 557.1986.

Determination of the configuration of sugars in **4-5**: Each compound **4** and **5** (1 mg) was hydrolyzed with 2 M HCl (2 mL) for 18 h. After cooling, the mixture was diluted with 5 ml water, passed through an Amberlite IRA400 column, lyophilized and the residues obtained were converted to the corresponding thiocarbamoyl-thiazolidine carboxylate derivative with L-cysteine methyl ester and *O*-tolyl isothiocyanate as described above. The sugars were identified as L-rhamnose (*t*R, 21.32 min) and D-glucose (*t*R, 12.21 min) respectively from the hydrolysis experiments with **4** and **5** [authentic samples: D- rhamnose (*t*R, 11.73 min) and L- rhamnose (*t*R, 21.64 min); D-glucose (*t*R, 12.35) and L-glucose (*t*R, 11.12 min)] [12].

Sweetness Recognition Threshold (SRT) measurement of **1**: The sweetness recognition threshold of **1** was measured by three experienced panelists in duplicated runs. All solutions were made in carbon-treated water and used at room temperature. Each of three subjects were asked to isosweet the random, different order blind samples against standard sugar solutions at 0.5%, 1.0% and 1.5% (w/v). The subjects were asked to focus on second sip of each sample and to rinse their mouths with water in between samples. The blind results indicated both duplicated runs yielded consistent results among samples at three different concentrations of 35, 50 and 65 ppm of **1** and the overall % sweetness equivalence (SE) averages were 0.59, 0.92 and 1.06, respectively. As a result, the SRT at 0.75% SE of sugar in water is estimated to be 42 ppm for **1**. Similarly, the SRT of **1** in carbonated lemon-lime (LL) soda prototypes without sweetener was evaluated and determined to be 150 ppm.

Sweetness Enhancement Effect (SEE) measurement of **1**: In order to find the SEE, test solutions of glucose, fructose and sucrose were prepared equivalent to 6% SE with carbon-treated water at room temperature at SRT of **1** in each carbohydrate solution. The same 6% SE solutions with the above three carbohydrates were prepared without **1**. Experimental results indicated that **1** was found to have a slight, positive SEE (ca. 1% SE more) in glucose and fructose solutions whereas a relatively larger SEE in sucrose by ca. 2% SE compared to the solutions of glucose, fructose and sucrose without 1. Likewise, the SEE in carbonated lemon-lime (LL) soda prototypes was evaluated for sensory data in a trained, descriptive analysis of 10 descriptive analysis panelists by preparing 8% SE solution using high fructose corn syrup (HFCS), with and without **1** as described above. Each assessor evaluated all the beverage products in triplicate runs with 8 min break time between testing samples. Unsalted cracker, 0.75% saline solution and mineral grade water was used as a mouth rinse and refresher before testing each sample. Sensory data revealed that the 8% HFCS containing **1** had a SE of 9.2% which is 1.15 times as sweet as the prototype without **1**.

Conclusions

Five glycosides including three diterpene, a phenolic and a lignan were isolated from the commercial extract obtained from the leaves of *R. suavissimus* obtained from Chengdu Biopurify Phytochemicals Limited, China. The structures of all the isolated new compounds were identified as rubusoside (**1**), suavioside-A (**2**), sugeroside (**3**), quercetrin (**4**) and arctiin (**5**) on the basis of spectroscopic and chemical studies as well as by comparing their physical properties reported in the literature. This is the first report of the isolation of quercetrin

and arctiin from *R. suavissimus* in nature. The complete ^{1}H and ^{13}C NMR spectral assignments of all the isolated compounds are reported herewith in CD_5N_5 based on COSY, HSQC, HMBC, and MS/MS spectroscopic data as well as chemical studies. The sensory evaluation results demonstrated that the SRT of **1** in water and LL soda matrixes are 50 and 150 ppm respectively. Also, **1** showed ca.1% SSE in glucose and fructose, and ca.2% SSE in sucrose in aqueous solutions; whereas it showed 1.15 times SEE in LL soda prototypes at its SRT.

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