Sweet and Bitter Diterpene-Glucosides from Leaves of *Rubus suavissimus*

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From leaves of *Rubus suavissimus*, two minor ent-kaurane type diterpene glucosides were isolated in addition to the major sweet principle, rubusoside (1). Of these glucosides, the bitter glycoside (2) was identified as sugeroside which has been isolated from leaves of *Ilex sugerokii* var. *brevipedunculata etc.* The new glucoside (3) named suavioside-A tastes sweet and the structure was elucidated as 17-*O*-β-D-glucoside of ent-kaurane-3α,16β,17-triol.

Keywords *Rubus suavissimus*; Chinese plant resource; Rosaceae; sweet glucoside; diterpene glucoside; ent-kaurane-3α,16β,17-triol 17-*O*-β-D-glucoside; suavioside A; sugeroside

Previously, a sweet ent-kaurane-type diterpene glycoside named rubusoside (**1**, β-D-glucosyl ester or 13-*O*-β-D-glucosyl-steviol) was isolated in a high yield from leaves of a Chinese rosaceous sweet plant which grows in Kwangshi and Kwangton in the southern part of China.[1] In the first paper on the isolation of **1**, we tentatively described the name of this plant as *Rubus chingii* HU. However, further botanical and chemotaxonomical investigation led to the designation of this plant as *Rubus suavissimus* S. LEE (Chinese name: 甘葉懸鈎子).[2,3] Improvement of sweetness of **1** by enzymic transglucosylation was also reported.[4] This paper deals with the isolation of two minor diterpene glycosides from leaves of this plant.

From the water extract of the dried leaves, **1** was obtained in a high yield.[1] The mother liquor of the recrystallization of **1** from methanol was subjected to chromatography on silica gel followed by high performance liquid chromatography (HPLC) to give a bitter glucoside (**2**) and a sweet glucoside (**3**).

The X-ray crystallographic analysis of **2** led to the formulation of 17-*O*-β-D-glucoside of ent-kaurane-16β,17-diol-3-one which has already been isolated from leaves of *Ilex sugerokii* var. *brevipedunculata etc.*, otherwise named sugeroside.[5] Identification was further confirmed by comparison of the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum, mp and optical rotation.

The new sweet glucoside named suavioside A (**3**) yielded glucose on hydrolysis with β-D-glucosidase. The ^{13}C-NMR spectrum of **3** exhibited signals due to a β-glucopyranoside moiety attached to a primary alcohol.[6] Further, carbon signals of **3** were compared with those of **2** and the aglycone (**4**, ent-kaurane-3α,16α,17-triol) of aniseoside (**5**)[7] from *Operculina aurea* (Table I). In the spectrum of **3**, the signals due to the A-ring, and C-6 and -7 of **4** as well as those assignable to C- and D-ring carbons of **2** appeared at very similar positions. The ^{1}H-NMR spectrum of **3** exhibited a signal due to a carbinyl proton at δ 3.60 (1H, triplet-like, $W_{1/2}$ = 2.4 Hz) together with a pair of doublets [at δ 4.48 and 3.91 (J = 10.6 Hz)] which were observed for **2** at the similar chemical shifts. It follows that **3** can be formulated as 17-*O*-β-D-glucoside of ent-kaurane-3α,16β,17-triol (**6**).

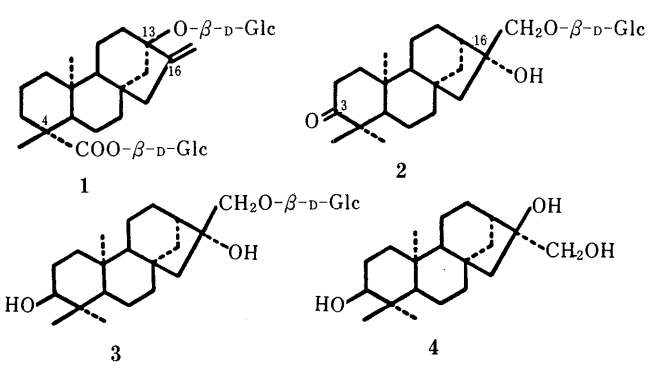

Chart 1

Experimental

General Procedure Optical rotations were measured with a Union PM-101 automatic digital polarimeter. NMR spectra were recorded on a JEOL GX-400 instrument in C_5D_5N, using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were taken on a JEOL SX-102 spectrometer.

TABLE I. ^{13}C-NMR Chemical Shifts of **2**—**4** (δ from TMS in C_5D_5N)

Carbon	2	3	4
Aglycone moiety			
1	39.2	33.8	33.9
2	34.3	27.0[a]	27.6[a]
3	216.7	75.2	75.1
4	47.2	38.1	38.6
5	54.3	49.0	49.0
6	21.9[a]	20.5[b]	19.1[b]
7	41.3	42.5	42.3
8	44.5	44.9	43.8
9	55.5	56.8	57.4
10	38.6	39.6	39.5
11	19.0[a]	18.6[b]	20.0[b]
12	26.6	26.5[a]	26.4[a]
13	46.3	46.6	41.8
14	37.1	37.6	38.0
15	52.9	53.4	53.55
16	80.8	80.9	79.6
17	75.5	75.7	70.35
18	27.3	29.4	29.3
19	21.1	22.4	22.4
20	17.8	18.0	17.8
Glucosyl moiety			
1	106.6	106.7	
2	75.5	75.6	
3	78.7[b]	78.6[c]	
4	71.7	71.7	
5	78.8[b]	78.7[c]	
6	62.9	62.9	

a—c) These assignments may be interchanged in each column.

Extraction and Separation of Glycosides The plant is cultivated at the Botanical Garden of South China Institute of Botany. The dried leaves (740 g) were extracted with hot water to give a water extract (192 g) which was treated with 95% EtOH to remove insoluble substances. The EtOH-soluble fraction was passed through a column of alumina and the eluate was recrystallized from MeOH to give **1** (26 g). The mother liquor was chromatographed on silica gel with EtOAc–MeOH–H_2O (60 : 5 : 1) to give a mixture of **2** and **3** which was separated by HPLC, affording **2** and **3** in yields of 0.01 and 0.006%, respectively. Condition of HPLC; column: ODS-120T (21.5 mm × 30 cm, Toso Co., Ltd.), mobile phase: 31% CH_3CN, flow rate: 6 ml/min, detection: differential refractometer.

2: Colorless needles from MeOH, mp 209—211 °C, $[\alpha]_D^{23}$ −55.6° ($c=0.36$, C_5H_5N). HR-FAB-MS: Calcd for $[C_{26}H_{43}O_8+H]^+$; 483.2958. Found: 483.2930 $[M+H]^+$. ^{1}H-NMR δ: 5.05 (1H, d, $J=7.7$ Hz anomeric H), 4.54, 3.95 (each 1H, d, $J=10.7$ Hz, 17-CH_2), 1.09, 1.02, 0.93 (each 3H, s). X-Ray crystallography of **2**: The crystals used for the X-ray crystallography were obtained as a water solvate and had dimensions of 0.3 × 0.2 × 0.2 mm. The crystal data are as follows: $C_{26}H_{42}O_8 \cdot H_2O$, $M_r=500.6$, monoclinic, space group $P2_1$, $a=14.915(2)$, $b=13.498(2)$, $c=6.131(1)$Å, $\beta=92.69(3)$, $V=1232.9$ Å^3, $Z=2$, $D_x=1.348$ g cm^{-3}. Intensity data were recorded on a Rigaku AFC-5R diffractometer using graphite-monochromated MoK_α radiation ($\lambda=0.71069$ Å). Out of a total of 3738 independent reflections within a 2θ range from 2° to 60°, 1922 reflections $[F_O \geqq 3\sigma(F_O)]$ were considered as observed and used for the structure determination. The crystal structure was solved by the direct method and refined by block-diagonal least-squares calculations. The final R value was 0.058.

3: A white powder, $[\alpha]_D^{25}$ +47.21° ($c=0.14$, C_5H_5N). HR-FAB-MS: Calcd for $[C_{26}H_{45}O_8+H]^+$; 485.3114. Found: 485.3193 $[M+H]^+$. ^{1}H-NMR δ: 5.04 (1H, d, $J=7.9$ Hz, anomeric H), 4.48, 3.91 (each 1H, d, $J=10.6$ Hz, 17-CH_2), 3.60 (1H, triplet-like, $W_{1/2}=2.4$ Hz, 3-CH). A solution of **2** (1 mg) and β-D-glucosidase from almonds (2 mg) in H_2O (1 ml) was incubated at 37 °C for 5 d. Glucose was identified in the reaction mixture by thin-layer chromatography (TLC) on silica gel developed with $CHCl_3$–MeOH–H_2O (6 : 4 : 1); detection: 2,3,5-triphenyltetrazolium chloride reagent.

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