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19α-Hydroxyursane-Type Triterpene Glucosyl Esters from the Roots of *Rubus suavissimus* S. LEE[1)]

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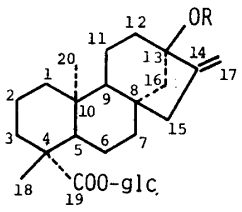
No diterpene glycoside was isolated from the roots of *Rubus suavissimus* S. LEE, in contrast to its leaves, which taste sweet and contain a large amount of the sweet diterpene glucoside named rubusoside. Instead, a new 28-β-gluchopyranosyl ester of 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid named suavissimoside F1 was isolated from the roots of this plant, along with niga-ichigoside F1 (28-β-glucopyranosyl ester of 19α-hydroxyasiatic acid) which has already been obtained from leaves of other *Rubus* spp.

Keywords——*Rubus suavissimus* root; Rosaceae; 19α-hydroxyursolic acid derivative; triterpene 28-β-glucopyranosyl ester; niga-ichigoside F1; suavissimoside F1

In our Chinese–Japanese cooperative studies on Chinese sweet plants, the sweet kaurane-type diterpene glucoside named rubusoside (**1**) was isolated in a yield of 5.4% from sweet leaves of a rosaceous plant collected in South China.[2)] This plant, which was first tentatively identified as *Rubus chingii* HU, has been now designated as *R. suavissimus* S. LEE.[3,4)] This is the first example of the isolation of a diterpene-glycoside from rosaceous plants and it is also noteworthy that **1** was reported to be an important intermediate for the synthesis of rebaudioside-A (**2**) from stevioside (**3**),[5)] both of which are sweet principles of *Stevia rebaudiana* BERTONI (Compositae). In connection with this study, several labdane-type diterpene glucosides were isolated instead of **1** (kaurane-type diterpene glucoside) from the leaves of *R. chingii* collected in Japan (Japanese name: Gosho-ichigo).[4)] Further, chemical

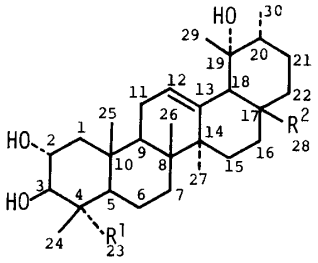


1: R = glc

2: R = glc(2→1)glc, glc(3→1)glc

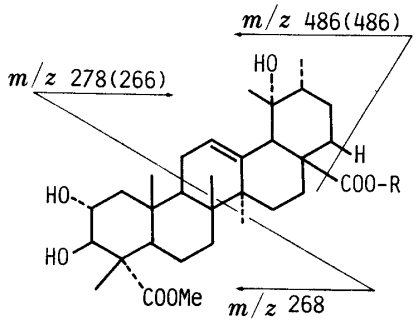
3: R = glc(2→1)glc

glc = β-D-glucopyranosyl



4: $R^1 = CH_2OH$, $R^2 = COOH$
5: $R^1 = CH_2OH$, $R^2 = COO$–glc
6: $R^1 = COOH$, $R^2 = COO$–glc
8: $R^1 = COOH$, $R^2 = COOH$
9: $R^1 = COOMe$, $R^2 = COOMe$
10: $R^1 = CH_2OH$, $R^2 = COOMe$
11: $R^1 = CH_2OH$, $R^2 = CH_2OH$

Chart 1



9: R = Me (**12**: R = H)

Chart 2. EI-MS Fragmentation

screening of the leaves of thirty-nine other *Rubus* spp. revealed that the presence of diterpene glucosides is limited to the leaves of *R. suavissimus* and *R. chingii*, and glucosyl esters of 19α-hydroxyursane-type triterpenes are more common as constituents of the leaves of *Rubus* spp.[6] As a continuation of our studies on *Rubus* spp., the present paper deals with the chemical investigation of glycosides of the roots of *R. suavissimus*, which do not taste sweet in contrast to the leaves. It was found that the roots contain triterpene glucosyl esters similar to those of the leaves of other *Rubus* spp.

A glycoside fraction of the roots was subjected to chromatography, affording three glucosides (**5**—**7**) in yields of 0.04, 0.17 and 0.15%, respectively. Of these, the least polar glucoside **5** was identified as the 28β-glucopyranosyl ester of **4**, which was previously isolated from the leaves of *R. microphyllus* L.F. and some other *Rubus* spp. and named niga-ichigoside F1.[6]

TABLE I. ^{13}C-NMR Chemical Shifts (in C_5D_5N)

Carbon	**6**	**8**	**9**	**10**	**11**
1	48.1	48.1	48.0	47.8	48.1
2	68.6	68.6	68.3	68.8	69.0
3	80.9	80.9	80.6	78.2	78.4
4	54.6	54.7	55.1	43.6	43.7
5	52.0	52.2	51.8	48.2	48.1
6	21.3	21.4	21.4	18.6	18.7
7	33.1	33.3	33.0	33.0	33.0
8	40.6	40.5	40.3	40.3	41.0
9	48.4	48.1	48.0	48.2	48.1
10	38.4	38.5	38.4	38.2	38.2
11	24.5	24.2	24.0	24.1	24.4
12	128.0	127.7	127.8	128.1	127.6
13	139.2	139.9	139.3	139.4	140.2
14	42.0	42.0	41.9	42.1	42.0
15	28.9	29.1	28.8	28.9	27.6
16	25.8[a]	26.3[a]	25.9[a]	26.8[a]	25.7[a]
17	48.4	48.6	48.5	48.2	38.8
18	54.6	54.7	54.4	54.4	55.7
19	72.6	72.7	72.5	72.5	73.5
20	42.0	42.0	42.1	42.1	42.8
21	26.3[a]	27.1[a]	26.7[a]	27.2[a]	27.1[a]
22	37.6	38.5	38.1	38.2	36.3
23	180.0	180.6[b]	178.0[b]	66.5	66.7
24	13.3	13.4	13.1	14.3	14.4
25	17.3[b]	17.3[c]	17.3[c]	17.3[b]	17.6[b]
26	17.3[b]	17.1[c]	16.7[c]	17.3[b]	17.1[b]
27	24.5	24.7	24.6	24.6	24.4
28	176.8	180.0[b]	178.3[b]	178.4	69.4
29	26.9	27.1	26.9	27.2	27.1
30	16.6[b]	16.8[c]	16.7[c]	16.6[b]	17.1[b]
COOMe			52.4	51.4	
			51.5		
G-1	95.6				
2	73.9				
3	78.9				
4	71.2				
5	78.9				
6	62.3				

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

On hydrolysis with crude hesperidinase,[7] the new glucoside **6**, named suavissimoside R1, yielded glucose and an aglycone (**8**), which was crystallized as its dimethyl ester (**9**) after treatment with diazomethane. A comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **9** with that of the methyl ester (**10**) of 19α-hydroxyasiatic acid (Table I) showed that the signals due to C-3, -4, -5 and -6 of **10** were displaced downfield and the signal attributable to 23-CH_2OH of **10** was replaced by signals associated with $-COOCH_3$ on going to **9**, while other resonances of **10** were observed at almost the same positions as in the spectrum of **9**. This suggested that **8** can be formulated as 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid. The mass spectrum (MS) of **9** showed M^+ (*m/z* 546) and fragment ions at *m/z* 486 (546-$COOCH_3$–H), 414, 268 and 278 (Chart 2), supporting this formulation, which was finally confirmed by conversion of **9** and **10** into the same pentaol (**11**) by reduction with $LiAlH_4$.

In the ^{13}C-NMR spectrum of **6** (Table I), a set of carbon signals[8] due to the β-glucopyranosyl ester moiety was observed.[6] Since no significant difference in chemical shift was noted between C-23 and -28 for **8** and **9** and glycosylation shifts are known to be limited to the signal of the glycosylated carboxyl carbon for glycosyl esters of this type,[6] it was impossible to determine whether the location of the β-glucopyranosyl ester linkage was at 23- or 28-COOH from the ^{13}C-NMR chemical shift. Accordingly, **6** was subjected to methylation with diazomethane followed by enzymic hydrolysis. The MS of the resulting monomethyl ester (**12**) of **8** exhibited M^+ (*m/z* 532) and fragment ions at *m/z* 486, 414 and 264 (Chart 2), indicating that the methyl ester group of **12** must be located at 23-COOH. It follows that **6** can be formulated as the 28β-glucopyranosyl ester of **8**. Very recently, isolation of **5** and **6** from *Geum japonicum* THUNB. (Rosaceae) was reported by Shigenaga *et al.*[9]

Although the most polar glycoside (**7**) seemed to be homogeneous in thin layer chromatography on silica gel, the ^{13}C-NMR spectrum revealed that **7** was still a mixture of closely related glycosides, the further separation of which is in progress. No diterpene glucoside such as **1** was detected in the roots.

Experimental

The NMR spectra were taken at 25.15 MHz for ^{13}C-NMR and at 90 MHz for ^{1}H-NMR. The MS were recorded at 75 eV (ionization voltage).

Plant Materials——Roots of *R. suavissimus* were collected in Guangxi and Guangzhou (the South China Botanical Garden, Academia Sinica). A specimen was authorized by F. Chen, Director of South China Institute of Botany, Academia Sinica, and is deposited in the Herbarium of this Institute, Guangzhou.

Extraction, Separation and Properties of Glucosides——The air-dried roots (500 g) were extracted with hot MeOH. The extract was evaporated to dryness, and a suspension of the resulting residue was washed with Et_2O then extracted with 1-BuOH (saturated with H_2O), and the BuOH-layer was concentrated to dryness. A solution of the residue in H_2O was chromatographed on a column of Diaion HP-20 (Mitsubishi Kasei Co., Ltd., Tokyo); elution was carried out with H_2O, 30% MeOH, 70% MeOH and MeOH successively. The fraction eluted with 70% MeOH was subjected to chromatography on silica gel, and elution with $CHCl_3$–MeOH–H_2O (50 : 10 : 1, 30 : 10 : 1 and then 6 : 4 : 1, all homogeneous) afforded **5**, **6** and **7** in yields of 0.04, 0.17 and 0.15%, respectively.

5: Colorless needles from MeOH–H_2O, mp 230—231 °C, $[\alpha]_D^{23}$ +11.2° (*c*=0.93, MeOH). The identification of **5** as niga-ichigoside F1[6] was confirmed by comparison of the ^{13}C-NMR and proton nuclear magnetic resonance (^{1}H-NMR) spectra in C_5D_5N and other physical constants with those of an authentic sample.

Suavissimoside R1 (**6**): Colorless needles from MeOH, mp 255 °C (dec.), $[\alpha]_D^{24}$ +19.1° (*c*=1.2, MeOH). *Anal.* Calcd for $C_{36}H_{54}O_{12}\cdot 2H_2O$: C, 60.48; H, 8.18. Found: C, 60.63; H, 8.28. ^{1}H-NMR (in C_5D_5N) δ: 1.10, 1.17, 1.20, 1.38, 1.61 and 1.71 (all 3H, s), 2.92 (1H, br s, H (18)) and 6.28 (1H, d, *J*=7 Hz, anomeric H), infrared (IR) (Nujol): 1690 (COOH) and 1725 (ester) cm^{-1}.

Hydrolysis of 6 and Properties of 8 and 9——Toluene (1 ml) was added to a solution of **6** (157 mg) and crude hesperidinase (157 mg, Tanabe Pharm. Ind. Co., Ltd., Osaka) in H_2O (80 ml). After being sonicated for 1 h, the mixture was incubated at 38 °C for 19 h and then extracted with EtOAc–1-BuOH (2 : 1). Glucose in the aqueous layer was detected by gas-liquid chromatography (GLC) as its trimethylsilyl ether in the usual way. The organic layer was concentrated to dryness, affording the aglycone (**8**) as a white powder almost quantitatively; this product was treated

with CH_2N_2 in Et_2O–MeOH overnight. After purification by column chromatography on silica gel with EtOAc–*n*-C_6H_{14} (3:2), **9** (110 mg) was obtained as colorless prisms from EtOAc–*n*-C_6H_{14}, mp 103—104 °C, $[\alpha]_D^{22}$ +31.3° (*c*= 0.97, MeOH). *Anal.* Calcd for $C_{32}H_{50}O_7$: C, 70.30; H, 9.22. Found: C, 70.15; H, 9.46. ^{1}H-NMR (in $CDCL_3$) δ: 0.67, 0.98, 1.03, 1.18, 1.22 and 1.27 (all 3H, s), 3.61 and 3.73 (both 3H, s, $COOCH_3$).

A solution of **9** (110 mg) in anhydrous Et_2O, was treated with $LiAlH_4$ (1.0 g), and the mixture was refluxed under stirring for 3.5 h. Excess reagent was decomposed with EtOAc, and the mixture was acidified by addition of aqueous 2% H_2SO_4 then extracted with EtOAc–1-BuOH. The organic layer was concentrated to dryness and the residue was chromatographed on silica gel with $CHCl_3$–MeOH (10:1) to give **11** as a white powder (reprecipitated from EtOAc), $[\alpha]_D^{21}$ +27.7° (*c*=1.1, MeOH). *Anal.* Calcd for $C_{30}H_{50}O_5$: C, 73.43; H, 10.27. Found: C, 73.39; H, 10.30. This product was identical with the pentaol obtained from **10** by reduction with $LiAlH_4$ in the same way as above. The identification was substantiated by thin layer chromatography (TLC) comparison on silica gel (solvent, $CHCl_3$–MeOH–H_2O (50:10:1); detection, H_2SO_4) and by comparison of the ^{13}C-NMR spectra (see Table I) of both compounds.

Methylation of 6 Followed by Enzymic Hydrolysis for MS— —Compound **6** (100 mg) was treated with CH_2N_2 in Et_2O–MeOH and the resulting methyl ester was hydrolyzed with crude hesperidinase in the same manner as described for the hydrolysis of **6**, yielding **12** (32 mg) as a white powder, $[\alpha]_D^{14}$ +30.3° (*c*=1.8, MeOH). *Anal.* Calcd for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found: C, 70.02; H, 9.15. ^{1}H-NMR (in C_5D_5N) δ: 3.65 (3H, s, 23-$COOCH_3$), which was subjected to MS.

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