A New Triterpene Glucosyl Ester from the Fruit of the Blackberry (*Rubus allegheniensis*)

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A new triterpene glucosyl ester, rubusside A, has been isolated from the fruit of the blackberry (*Rubus allegheniensis* PORT.) along with a known triterpene glucosyl ester, niga-ichigoside F1. The chemical structure of rubusside A was determined on the basis of spectroscopic data as well as chemical evidence.

Key words *Rubus allegheniensis*; triterpene glucosyl ester; butylidene; blackberry; Rosaceae; rubusside A

The blackberry (Rosaceae), genus *Rubus*, consists of a variable and complex group of plants and grows widely throughout the world, especially in the temperate parts of the northern hemisphere. The fruit is eaten fresh or as one of many processed products, *e.g.*, jams, jellies, and pastry fillings. Recently, the oxygen radical absorbance capacity (ORAC), total phenolic content, and total anthocyanin of fruits of blackberry were reported,[1,2] and the consumption of blackberry is expected to be associated with low incidences of various human diseases caused by active oxygen species and free radicals.

Rubus allegheniensis PORT., which is distributed from the northeastern region to the middle region of North America, is one native species of blackberry used to make cultivars by hybridization. The present paper describes the isolation and structure elucidation of a new triterpene glucosyl ester along with a known triterpene glycosyl ester, niga-ichigoside F1, from the MeOH extract of *R. allegheniensis* PORT.

The MeOH extract of the fruit of *R. allegheniensis* PORT. was successively subjected to Diaion HP 20 and silica gel column chromatography as well as HPLC on ODS to afford two triterpene glycosyl esters (**1**, **2**).

Compound **2** was identified as niga-ichigoside F1 based on its physical and spectral data.[3]

Compound **1**, trivially called rubusside A, was obtained as an amorphous powder. In the positive FAB-MS, **1** showed an $[M+Na]^+$ ion peak at *m/z* 743. The molecular formula of **1** was determined to be $C_{40}H_{64}O_{11}$ by high-resolution (HR) positive FAB-MS. The ^{1}H-NMR spectrum of **1** showed signals due to five tertiary methyl groups (δ 1.68, 1.42, 1.23, 1.17, 1.04), one secondary methyl group (δ 1.08), one primary methyl group (δ 0.86), one olefin proton (δ 5.54), and one anomeric proton (δ 6.30). The ^{13}C-NMR spectrum of **1** showed 40 carbon signals, including one ester carbonyl carbon (δ 177.0), one tri-substituted double bond system (δ 139.3, 128.2), one hemiacetal carbon (δ 103.1), one oxygenated methylene carbon (δ 78.4), two oxygenated methine carbons (δ 90.8, 64.9), one oxygenated quaternary carbon (δ 72.7), and one glucopyranosyl group (δ 95.9, 74.1, 79.0, 71.3, 79.3, 62.4). These ^{1}H- and ^{13}C-NMR signals were assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) spectra as shown in Tables 1 and 2, respectively, and it was assumed that **1** is attached by a butylidene group to **2**.

To confirm the above suggestion, **1** was hydrolyzed. On acidic hydrolysis, **1** gave a mixture of derivatives of aglycone (Ag) and glucose, which was identified as the D-form by GC analysis according to the method of Hara *et al.*[4] The former mixture was methylated with trimethylsilyldiazomethane and then subjected to HPLC to show the presence of 2α,3β,23-trihydroxy-urs-12,18-dien-28-oic acid methyl ester (**3**) and 2α,3β,23-trihydroxy-urs-12,19-dien-28-oic acid methyl ester (**4**), which were the same products arising from **2** by similar treatment as for **1**. Although Durham *et al.*[5] have reported the ^{13}C-NMR data of **3** and **4,** the data differ slightly from those in this study (Table 2). The connectivity of the butylidene group was confirmed by the HMBC and the ^{13}C-NMR spectra. The HMBC spectrum showed a cross-peak between H-23a of Ag and C-1 of the butylidene group (Fig. 1). In comparing the ^{13}C-NMR signals of **1** and **2**, the signals of C-3 of Ag and C-23 of Ag in **1** were shifted downfield by 12.4 ppm and 11.8 ppm, respectively, while, the signals of C-

Table 1. ^{1}H-NMR Spectral Data for Compound **1** (in C_5D_5N, 500 MHz)

Position	**1**	Position	**1**
1a	*ca.* 1.33	22a	1.87 ddd (4.5, 13.0, 13.0)
1b	2.26 dd (4.5, 13.0)	22b	*ca.* 2.07
2	4.18 ddd (4.5, 10.0, 10.5)	23a	3.86 d (10.5)
3	3.34 d (10.0)	23b	3.30 d (10.5)
5	1.01 br d (12.0)	24	1.23 s
6a	*ca.* 2.20	25	1.04 s
6b	*ca.* 1.35	26	1.17 s
7a	*ca.* 1.58	27	1.68 s
7b	*ca.* 1.37	29	1.42 s
9	*ca.* 1.97	30	1.08 d (6.5)
11a	*ca.* 2.10	1′	6.30 d (8.5)
11b	*ca.* 2.10	2′	4.24 dd (8.5, 9.0)
12	5.54 dd (3.0, 3.0)	3′	4.31 dd (9.0, 9.0)
15a	2.47 ddd (4.5, 13.5, 14.0)	4′	4.36 dd (9.0, 9.0)
15b	*ca.* 1.24	5′	4.07 ddd (2.5, 4.5, 9.0)
16a	3.11 ddd (4.5, 13.0, 13.5)	6′a	4.41 dd (4.5, 12.0)
16b	*ca.* 2.03	6′b	4.49 dd (2.5, 12.0)
18	2.94 s	1″	4.75 dd (5.0, 5.0)
20	*ca.* 1.33	2″	1.76 m
21a	*ca.* 1.23	3″	1.53 tq (7.5, 7.5)
21b	*ca.* 1.23	4″	0.86 t (7.5)

δ in ppm from tetramethylsilane (coupling constants [*J*] in Hz are given in parentheses).

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Table 2. ^{13}C-NMR Spectral Data for Compounds **1**—**4** (125 MHz)

Position	**1**	**2**	**3**	**3**[a)]	**4**	**4**[a)]
1	48.8	47.9	46.7	46.6	46.5	46.8
2	64.9	68.9	68.9	68.7	68.8	68.7
3	90.8	78.4	80.9	79.4	81.0	79.9
4	37.3	43.6	42.4	42.5	42.3	42.4
5	51.6	48.0	49.6	48.6	49.7	48.9
6	17.5	18.7	18.4	18.5	18.3	18.1
7	33.0	33.2	34.3	34.0	33.4[b)]	33.2
8	40.6	40.7	39.0	38.8	39.2	39.1
9	47.8	47.9	47.8	47.5	47.7	47.5
10	38.4	38.4	38.2	37.9	38.2	38.0
11	24.0	24.2	23.3	31.0	23.3	23.1
12	128.2	128.4	125.4	125.2	126.8	126.6
13	139.3	139.3	138.7	138.5	137.7	137.5
14	42.2	42.2	44.7	34.8	43.4	43.2
15	29.2	29.2	28.5	28.3	28.3	28.2
16	26.1	26.1	26.6	21.7	21.9	21.8
17	48.6	48.6	49.5	49.3	47.0	46.4
18	54.5	54.4	132.8	135.9	50.0	49.8
19	72.7	72.7	136.1	132.6	128.2	128.0
20	42.2	42.1	34.3	44.5	123.8	123.6
21	26.7	26.7	31.2	26.3	28.0	32.5
22	37.7	37.7	35.0	34.1	32.7[b)]	27.8
23	78.4	66.6	71.3	69.1	71.3	69.8
24	14.9	14.3	12.8	12.9	12.8	12.8
25	18.0	17.5	17.9[b)]	18.1	17.5[c)]	17.4
26	17.4	17.5	17.8[b)]	17.7	16.9[c)]	16.8
27	24.6	24.5	21.8	23.1	23.4	23.3
28	177.0	177.0	177.0	177.0	178.0	177.9
29	27.0	27.0	19.3	19.2	17.6[c)]	20.2
30	16.7	16.7	18.7[b)]	17.7	20.4	17.5
OCH_3			51.6	51.5	51.6	51.5
1′	95.9	95.8				
2′	74.1	74.0				
3′	79.0	78.9				
4′	71.3	71.3				
5′	79.3	79.2				
6′	62.4	62.4				
1″	103.1					
2″	37.4					
3″	17.5					
4″	14.3					

a) 62.5 MHz.[5)] *b*, *c*) Assignments may be interchanged in the same column. **1**, **2**: In C_5D_5N. **3**, **4**: In $CDCl_3$.

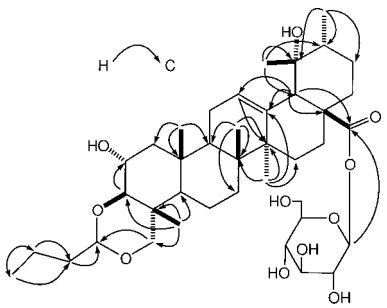

Fig. 1. ^{1}H–^{13}C Long-Range Correlations Observed for **1** in the HMBC Spectrum (in C_5D_5N, 500 MHz)

2 of Ag and C-4 of Ag were shifted upfield by 4.0 ppm and 6.3 ppm, respectively. These observations indicate that the butylidene group is attached to C-3 and C-23. The structure of **1** was therefore defined as 3,23-*O*-butylidene-2α,3β,19α,23-tetrahydroxy-urs-12-en-28-oic acid β-D-glucopyranosyl ester (Fig. 2). However, the configuration of the butylidene group was not determined.

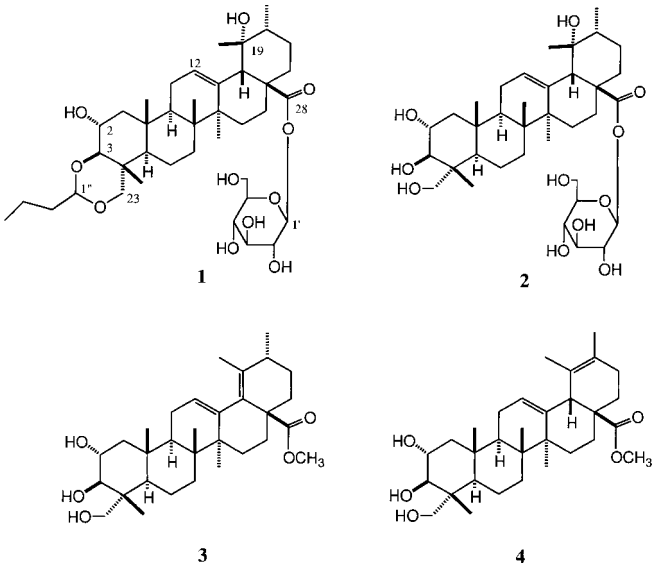

Fig. 2. Structures of **1**—**4**

As far as we know, **1** is a new glucosyl ester, and the isolation of **2**, which exhibited a potent antinociceptive effect,[6)] from the fruit of *Rubus allegheniensis* Port. is described here for the first time. However, it is possible that **1** is artifact formed from **2** during the concentration procedure, in which BuOH was used as an antifoaming agent.

Experimental

All the instruments and the materials used were the same as cited in the previous reports,[7,8)] unless otherwise specified.

Plant Material The fruit of *R. allegheniensis* Port. was collected in August 2000 from the orchard of Kyushu Tokai University, Kumamoto prefecture, Japan, and identified by Professor Haruki Komatsu, Kyushu Tokai University School of Agriculture.

Extraction and Isolation Crushed fruit of *R. allegheniensis* Port. (1408 g) was soaked in MeOH (1408 ml) for 24 h at room temperature (this procedure was repeated two times), and the solvent was removed under reduced pressure to give a syrup (118.8 g). The MeOH extract (108.8 g) was subjected to Diaion HP 20 (H_2O, 10% MeOH, 30% MeOH, 50% MeOH, 60% MeOH, 70% MeOH, 90% MeOH, MeOH, acetone) to give fractions (frs.) 1—9. Fr. 6 (682 mg) eluted with 70% MeOH was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (20 : 1 : 0, 10 : 1 : 0, 14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2)] to afford frs. 10—24. Fr. 15 (13 mg) was subjected to HPLC (column, COSMOSIL 5C18 AR-II, Nacalai Tesque, 250 mm×20 mm i.d.; solvent, 80% MeOH) to give **1** (3 mg). HPLC (COSMOSIL 5C18 AR-II, 55% MeOH) of fr. 19 (96 mg) afforded **2** (29 mg).

Rubusside A (**1**): Amorphous powder. $[\alpha]_D^{26}$ +30.5° (c=0.2, MeOH). HR positive FAB-MS *m/z*: 743.4320 $[M+Na]^+$ (Calcd for $C_{40}H_{64}O_{11}Na$: 743.4346). Positive FAB-MS *m/z*: 743 $[M+Na]^+$. Negative FAB-MS *m/z*: 557 $[M-162$ (hexose unit)$-H]^-$. ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Acidic Hydrolysis of 1 and 2 Compounds **1** (1 mg) and **2** (19 mg) in 2 mol/l HCl were each heated at 95 °C for 120 min. The reaction mixture was neutralized with 2 mol/l NaOH and then extracted with AcOEt. The aqueous layer derived from **1** was evaporated under reduced pressure to give a residue, which was converted into trimethylsilyl ether of the methyl thiazolidine 4(*R*)-carboxylate derivative and subjected to GC analysis (detector, FID; column, silicone OV-1, 30 m×0.32 mm i.d., Ohio Valley Specialty Chemical; column temperature, 230 °C; injector temperature, 270 °C; detector temperature, 280 °C; carrier gas, He; flow rate, 0.8 ml/min).[4)] The retention time (16.0 min) of this product was identical with that of an authentic sample of D-glucose derivative. The AcOEt extracts derived from **1** and **2** in MeOH were each treated with trimethylsilyldiazomethane. HPLC (COSMOSIL 5C18 AR-II, 85% MeOH) of the methylated mixture derived from **2** gave **3** (2 mg) and **4** (1 mg). The mixture derived from **1** exhibited the pres-

ence of **3** (t_R 13.1 min) and **4** (t_R 15.5 min) on HPLC analysis (detector: refractive index; column, Mightysil RP-18 [H] GP, Kanto Chemical, 250 mm×4.6 mm i.d.; solvent, 85% MeOH; column temperature, 35 °C; flow rate, 0.8 ml/min).

2α,3β,23-Trihydroxy-urs-12,18-dien-28-oic Acid Methyl Ester (**3**): Amorphous powder. ^{1}H-NMR spectral data (in $CDCl_3$, 500 MHz) δ: 5.36 (1H, dd, J=4.0, 4.0 Hz, H-12), 3.78 (1H, ddd, J=4.5, 9.5, 11.0 Hz, H-2), 3.69 (1H, d, J=11.0 Hz, H-3), 3.61 (3H, s, OCH_3), 3.44 (1H, d, J=10.0 Hz, Ha-23), 3.43 (1H, d, J=10.0 Hz, Hb-23), 1.71 (3H, s), 1.08 (3H, s), 1.07 (3H, d, J=7.5 Hz, H_3-20), 0.96 (3H, s), 0.91 (3H, s), 0.87 (3H, s). ^{13}C-NMR spectral data: see Table 2.

2α,3β,23-Trihydroxy-urs-12,19-dien-28-oic Acid Methyl Ester (**4**): Amorphous powder. ^{1}H-NMR spectral data (in $CDCl_3$, 500 MHz) δ: 5.48 (1H, dd, J=3.5, 3.5 Hz, H-12), 3.77 (1H, ddd, J=4.5, 9.5, 11.0 Hz, H-2), 3.68 (1H, d, J=11.0 Hz, H-3), 3.63 (3H, s, OCH_3), 3.44 (1H, d, J=10.0 Hz, Ha-23), 3.42 (1H, d, J=10.0 Hz, Hb-23), 1.62 (3H, s), 1.52 (3H, s), 1.07 (3H, s), 0.96 (3H, s), 0.90 (3H, s), 0.82 (3H, s). ^{13}C-NMR spectral data: see Table 2.

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