Phytochemistry, Vol. 29, No. 10, pp 3275–3280, 1990
Printed in Great Britain

0031-9422/90 $3.00+0.00

A DIMERIC TRITERPENE-GLYCOSIDE FROM *RUBUS COREANUS*

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(*Received* 20 *February* 1990)

Key Word Index—*Rubus coreanus*; Rosaceae; leaves; Bog-bun-ja; Fu-pen-zi; dimeric triterpene glucosyl ester; ursane-type triterpene; coreanoside F1.

Abstract—A dimer of glucosyl esters of A-ring oxygenated 19α-hydroxyursolic acids was isolated from leaves of *Rubus coreanus* together with the monomers of the related glucosyl esters. The structure of this compound, named coreanoside F1, was elucidated by chemical and spectroscopic methods. The significance of coreanoside F1 in the identification of the source plants of an oriental traditional medicine 'Bog-bun-ja' (= Fu-pen-zi) is discussed

INTRODUCTION

In Korea and China, fruits of some of *Rubus* spp. have been used as a traditional medicine (Korean name 'Bog-bun-ja', Chinese name 'Fu-pen-zi). As part of our studies on glycosides from *Rubus* spp. [1], the present paper deals with isolation and structure determination of a dimeric triterpene glycosyl ester from *R. coreanus* Miq. The significance of the present study in the chemical identification of source plants of this crude drug is also reported.

RESULTS AND DISCUSSION

A methanolic extract of leaves of *R. coreanus* cultivated in Toyama, Japan, was separated as described in the Experimental, affording four glycosides, **1**–**4** in yields of 0.02, 0.014, 0.42 and 0.33%. Compounds **1**–**3** were identified as the known β-glucosyl esters of A-ring oxygenated 19α-hydroxyursolic acids; **1** and **2**: niga-ichigosides F1 and F2, respectively, isolated from leaves of *R. microphyllus* L.f. and several other *Rubus* spp. [2], **3**: suavissimoside R1 isolated from roots of *R. suavissimus* S. Lee [3].

The molecular formula of a new glycoside (**4**) named coreanoside F1, was determined as $C_{72}H_{110}O_{23}$ by high resolution fast atom bombardment mass spectrometry (HR-FABMS). Acid hydrolysis of **4** yielded D-glucose, while hydrolysis of **4** with crude pectinase afforded an aglycone named coreanogenoic acid (**5**) together with D-glucose. On treatment with diazomethane, compound **5** yielded a trimethyl ester (**6**) and the HR-FABMS of **6** and the ^{13}C NMR spectrum of **5** and **6** (Table 1) demonstrated that **5** must be a dimeric triterpene acid.

Mild alkaline saponification of **4** yielded two acidic compounds, **7** and **8**, which are respectively treated with diazomethane to give corresponding dimethyl esters, **9** and **10**. By comparison of the NMR spectra and optical rotations of **7** and **9** with those of respective authentic samples, **7** was identified as 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid, the aglycone of **3** [2].

All of the signals assigned to the C, D and E-ring carbons of **9** appeared at almost the same positions in the ^{13}C NMR spectrum of **10**. This indicated that **8** is also a derivative of 19α-hydroxyursolic acid having no additional oxygen function on the C, D and E-rings. The EI mass spectrum of a triacetate (**11**) of **10** exhibited fragment ions, *m/z* 278 (Fig. 1) and *m/z* 260 (278-H_2O) which are characteristic of retro-Diels–Alder cleavage of the C-ring of 19-hydroxyursolic acid derivatives having no additional function on the D- and E-rings. Assignment of the ^{1}H and ^{13}C NMR signals of **10** by means of the ^{1}H–^{1}H and ^{13}C–^{1}H COSY coupled with ^{13}C–^{1}H long range COSY revealed that **10** is a dimethyl ester of 2,3,19α,23(or 24)-tetrahydroxyurs-12-ene-24(or 23),28-dioic acid (**7**). The configurations of the functional groups on the A-ring of **8** were elucidated by the more detailed NMR analysis of **11**. The ^{1}H NMR spectrum of **11** exhibited signals due to two protons on vicinal carbons bearing an acetoxyl group at δ5.73 (1H, *d*, *J* = 2.8 Hz) and 5.55 (1H, *ddd*, *J* = 2.8, 4.8, 9.9 Hz), disclosing the presence of 2α,3α-diacetoxyl groups. In the NOE difference spectrum of **11**, on irradiation of the H-2 signal, NOE was observed not only for H-3 and 25-methyl signals but also for a signal assignable to the carbomethoxy-protons (at δ3.62, 3H, *s*) attached to C-4 (Fig. 2), while no NOE was observed between the H-2 signal and signals due to acetoxymethylene protons at δ4.27 and 4.33 (each 1H, *d*, *J* = 10.3 Hz). It follows that **7** can be formulated as 2α,3α,19α,23-tetrahydroxyurs-12-ene-24,28-dioic acid. The possibility of a boat conformation of the A-ring was excluded.

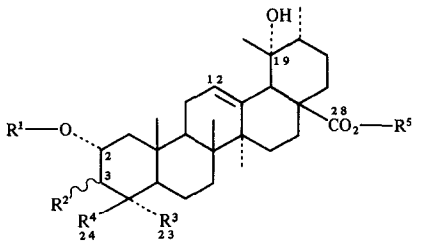
Location of an ester linkage which connects **7** with **8** in compound **5**, was elucidated by the acylation shift as well as the long range ^{13}C–^{1}H coupling in the NMR spectra. On going from **10** to **6**, the signal due to H-3′ of the **8**-moiety at δ5.22 (1H, *br s*) was displaced downfield by 1.36 ppm [at δ6.58 (1H, *br s*)], while other carbinyl proton signals remained almost unshifted (Table 2). The acylation shift was also observed for the carbons around C-3′ of the **8**-moiety on going from **10** to **6** (Table 1). The

Table 1 ^{13}C NMR spectral data of aglycone moieties of compounds **4–10**, **12** and **13** (pyridine-d_5, δ values)

C	**4**	**5**	**6**	**12**	**13**	**7**	**9**	**8**	**10**
1	48.2	48.2	48 2	48 3	48.3	48.1	48.0		
1′	44.7	44 5	44 2	44 3	44.3			43.4	43.4
2	68.4	68 1	68.0	68 1	68.1	68 5	68.4		
2′	66.1	67 7	65 3	65 4	65 4			66.2	66 1
3	82 0	82 0	82.1	82 2	82.1	80.9	80.7		
3′	74 1	75 0	73 4	73 4	73 4			70.4	70 3
4	54 8	54.7	54 8	54.8	54.8	54.7	55.2		
4′	55.8	55 8	55 9	55 9	55 8			55.4	55.1
5	51 3	51 3	51 5	51 4	51.4	52 2	51 8		
5′	48.1	48 1	48 1	48.2	48.2			48 1	47.1
6	21 9	21 6	21 6	21 7	21.6	21 4	21 4		
6′	20.1	20 7	20 4	20 6	20.5			20 7	20.4
7	33.1	33 1	32.8	32 9	32.9	33.3	33 2		
7′	34.0	33 7	33 5	33 7	33 7			33 6	33 4
8	40 7	40 6	40 4	40 7	40 6	40 5	40.4		
8′	40.2	40 4	40 1	40.4	40.4			40.3	40.2
9	48.3	48 3	47 1	47.2	47 2	48 1	48 0		
9′	48.1	48 1	47 3	47.5	47.7			46 2	45 3
10	38.2	38 2	38.1	38.2	38.2	38.5	38 4		
10′	39 2	39 2	38 8	38 8	38 9			39 1	38 9
11	24 2	24 1	23 9	24.1	24 1	24.2	24 1		
11′	24 4	24.3	24 1	24.2	24 2			24 3	24 2
12	128 3	127 9	128 0	128 1	127 9	127 7	127 7		
12′	128 9	128 3	128 2	128 4	128 2			128 2	128 3
13	139 4	139 8	139.5	139.4	139 9	139 9	139 3		
13′	139 3	139 8	139 4	139 2	139 8			139 8	139 4
14	42 1	42 2	42 0	42 2	42 2	42 4	42 2		
14′	42 3	42 3	41 9	42 1	42 3			42 1	42 1
15	29 1	29 1	28 8	29 1	29 1	29 0	29 2		
15′	29 2	29 2	28 9	29 2	29 2			29 1	28 9
16	26 2	26 5	26 0	26 1	26 1	26 3	25 9		
16′	26 3	26 4	26 0	26 0	26 0			26 1	26 1
17	48 3	48 3	48 5	48 5	48 6	48 6	48 5		
17′	48 3	48.3	48 6	48 7	48 7			48 6	48 6
18	54 6	54.6	54 5	54 5	54 5	54 6	54 5		
18′	54 3	54 3	54 3	54 3	54 4			54 5	54 5
19	73 0	73.0	72 7	72 8	72 9	72 7	72 7		
19′	73 0	73 0	72 7	72 8	72 9			72.7	72 6
20	42 2	42 2	42 1	42.1	42 1	42 0	42 1		
20′	42 3	42 3	42 2	42 2	42 2			42 1	42 2
21	26 7	27 3	26 6	26 7	27 3	27 1	26 9		
21′	26 7	27 4	26 6	26 7	27 4			27 4	26 7
22	37 6	38 3	38 0	37 7	38.5	38 5	38 1		
22′	37 6	38 3	38 0	37 7	38 0			38 0	38 1
23	178 5	178 2	177 7	177 8	178 2	178 0	178 3		
(OMe)							51 4		
23′	66 1	65 8	66 5	66 6	65 9			67 0	66 8
24	12 5	12 5	12 6	12 6	12.5	13 4	13 1		
24′	176.9	180 5	175 2	175 2	175 4			178 5	175 5
(OMe)			51 2	51 2	51 1				51 1
25	17 5	17 5	17 6	17 7	17 5	17.3	17 3		
25′	15 4	15 1	14 7	14.8	14 8			14 8	14 7
26	17 5	17 3	17 0	17 5	17 4	17 0	17 0		
26′	17 6	17 3	17 0	17 4	17 4			17 0	17 0
27	24.5	24 7	24 5	24 4	24 6	24.7	24 6		
27′	24.6	24.8	24 5	24 5	24 5			24 5	24 4
28	176 8	180 4	178 4	176 9	180 5	180 6	178 3		
(OMe)			51 6				51 8		
28′	176 8	180 4	178 4	176 9	180 5			180 4	178 5
(OMe)			51.7						51 6
29	27 2	27 3	27 0	27 2	27 1	27 1	27 0		
29′	27 3	27 4	27 1	27 1	27 0			27 0	26 9
30	16 7	16 7	16 7	16 7	16 8	16 8	16 8		
30′	16 6	16 6	16 6	16 7	16 8			16 6	16 6

long range coupling between signals assignable to C-23 of the 7-moiety (δ178.4) and to H-3′ (δ6.58) of the 8-moiety was substantiated in the spectrum of **6** by means of COLOC procedure. Further, on going from **9** to **6**, the carbon signal due to the 24-methyl carbon of the 7-moiety was displaced upfield by 1 ppm. These results indicated that the ester linkage in **5** must be located between 3′-OH of **8** and the 23-carboxyl group of **7**.

It is known that in comparison with alkyl glucosides and glucosyl-glycosides, an anomeric carbon signal of ester type glycosides appears at significantly higher field (δ95–96 in pyridine-d_5) and an anomeric proton signal of glucosides of this type is observed at remarkably low field (lower than δ6.0 in pyridine-d_5). The ^{1}H and ^{13}C NMR spectra of **4** (Tables 1 and 2) indicated the presence of two ester type β-glucopyranosyl moieties [2]. The anomeric configuration of both the glucosyl moieties was substantiated by the coupling constant of the anomeric proton signals as well as chemical shifts of the sugar carbon signals. Location of these two glucosyl groups in **4** was elucidated as follows. Treatment of **4** with diazomethane yielded a monomethyl ester (**12**), which by hydrolysis with crude hesperidinase, gave a monomethyl ester (**13**) of compound **5**. In the NOE difference spectrum of **13**, on irradiation of a carbomethoxy proton signal, NOE was observed for signals due to protons of C-2′, C-23′ and C-25′ of the **8**-moiety (Fig. 3). This indicated that both the β-D-glucosyl moieties must be located at 28- and 28′-carboxyl groups, leading to the structure of **4** (Chart 2). From leaves of *R. coreanus* cultivated in Seoul, Korea, compounds **1**–**4** were isolated in similar yields to those of the leaves cultivated in Japan.



	R¹	R²	R³	R⁴	R⁵
1	H	β-OH	CH_2OH	Me	β-D-Glc
2	H	α-OH	CH_2OH	Me	β-D-Glc
3	H	β-OH	CO_2H	Me	β-D-Glc
7	H	β-OH	CO_2H	Me	H
8	H	α-OH	CH_2OH	CO_2H	H
9	H	β-OH	CH_2OH	Me	Me
10	H	α-OH	CH_2OH	CO_2Me	Me
11	Ac	α-OAc	CH_2OAc	CO_2Me	Me

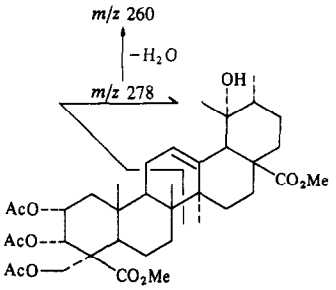


Fig. 1. EIMS fragmentation of compound **11**.

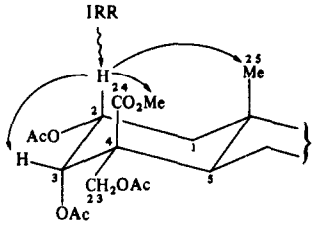
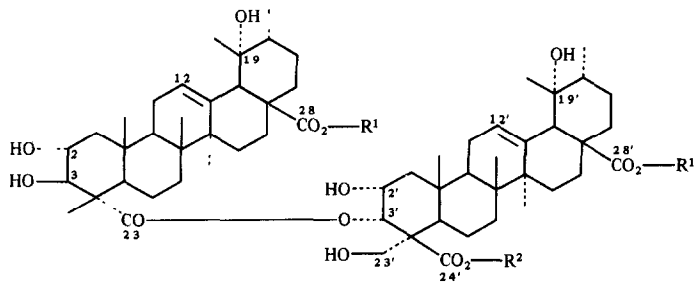


Fig. 2 NOEs observed in NOEDS of compound **11**

In China and Korea, fruits of some of *Rubus* spp. have been used as a tonic for aged people. The source plants of this drug have been stated as *R. chingii* Hu, *R. foliolosus* D. Don., *R. coreanus* Miq., *R. crategifolius* Linn., *R. parvifolius* Bunge. etc. We have isolated kaurane-type sweet diterpene-glycosides named rubusoside [4] and suavioside A[5] from leaves of *R. suavissimus* S. Lee [6] collected in Kuang-xi and Kuang-dong, China*. Several

*In our first paper [4] of isolation of rubusoside, the sweet plant was tentatively designated as *R. chingii* Hu. However, leaves of *R. chingii* collected in Japan and the other part of China do not taste sweet. The subsequent chemotaxonomical study on *Rubus* spp. revealed the difference between *R chingii* and this sweet plant, the latter of which has been designated as the new sp, *R suavissimus* S. Lee [6].



	R¹	R²
4	β-D-Glc	H
5	H	H
6	Me	Me
12	β-D-Glc	Me
13	H	Me

Table 2 ^{1}H NMR spectral data of compounds **6**, **9**–**11** (pyridine-d_5, **4** in $CDCl_3$, at 40°, δ values)

H	6	9	10	11
2	4 17 *ddd* (4 2, 9 5, 10 7)	4 18 *ddd* (4 2, 9 5, 10 5)		
2′	4 88 *br d* (10 7)		4 83 *br d* (10 2)	5 55 *ddd* (2 8, 4 8, 9 9)
3	4 38 *d* (9 5)	3 82 *d* (9 5)		
3′	6.56 *br s*		5 22 *br s*	5 73 *d* (2 8)
12	5 37 *t*-like	5 45 *dd* (3 3, 3.5)		
12′	5 39 *t*-like		5 56 *dd* (3 4, 3 6)	5 34 *dd* (3 4, 3 5)
18	2 77 *s*	2 78 *s*		
18′	2.76 *s*		2 81 *s*	2 62 *s*
H_2-23′	4 03, 4 49 *d* (10 3)		4 53, 4 58 *d* (10 3)	4 27, 4 33 *d* (10 3)
H_3-24	1 58 *s*	1 38 *s*		
H_3-25	1 08 *s*	0 98 *s*		
H_3-25′	0 96 *s*		1 03 *s*	0 72 *s*
H_3-26	0 83 *s*	0 83 *s*		
H_3-26′	0 89 *s*		0 89 *s*	0 87 *s*
H_3-27	1 76 *s*	1 58 *s*		
H_3-27′	1 56 *s*		1 58 *s*	1 32 *s*
H_3-29	1 27 *s*	1 29 *s*		
H_3-29′	1 35 *s*		1 37 *s*	1 27 *s*
H_3-30	1 09 *d* (6 7)	1 06 *d* (6 7)		
H_3-30′	1 06 *d* (6 7)		1 07 *d* (6 7)	0 94 *d* (6 5)
23-OMe		3 68 *s*		
24′-OMe	3 58 *s*		3 62 *s*	3 58 *s*
28-OMe	3 71 *s*	3 72 *s*		
28′-OMe	3 68 *s*		3 71 *s*	3 76 *s*
Ac				1 95 *s*
				1 96 *s*
				2 07 *s*

The values in parentheses are coupling constants in Hz

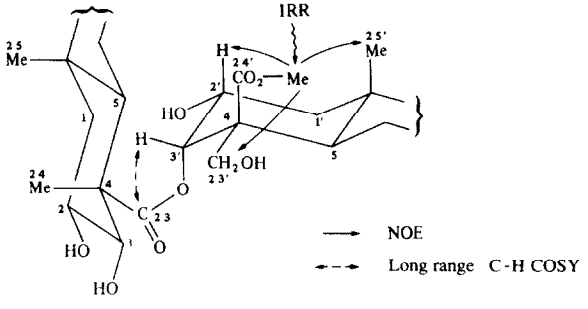


Fig 3 NOEs observed in NOEDS and long range C–H correlation of compound **13**

non-sweet labdane-type diterpene-glycosides named goshonosides were isolated from leaves and fruits of Japanese [7] and Chinese [1] *R. chingii* Hu which is used as a source plant of Fu-pen-zi in southern China. It is notable that *R. chingii* is morphologically very similar to *R suavissimus* Very recently, goshonosides and their homologues were isolated also from fruits of *R. foliolosus* D. Don [8], which are morphologically different from fruits of *R chingii* but have also been used as Fu-pen-zi in South-Western China (Yunnan). From fruits and leaves of these three *Rubus* spp. (tentatively named group A), no triterpene glycoside has been isolated

Chemical identification of source plants (group A) of Chinese Fu-pen-zi used in southern China was studied by

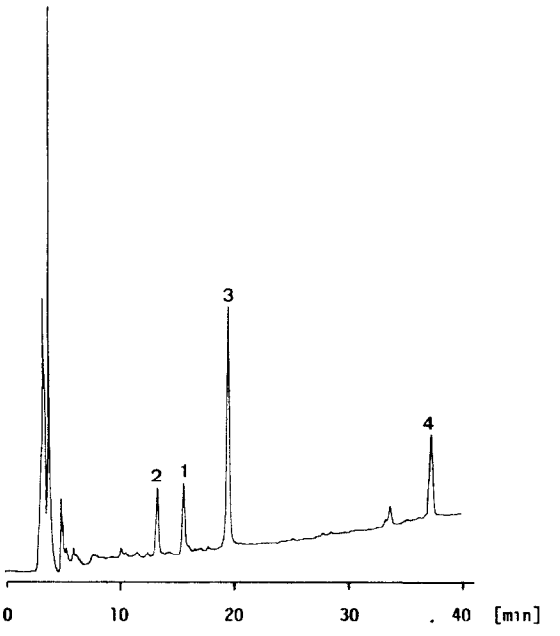


Fig 4 HPLC chromatogram of glycosides fraction from commercial 'Bog-bun-ja' on a TSKgel Amide-80 column Column size, 25 cm × 4 0 mm, column temp, 60°, mobile phase, MeCN–0.05% TFA [37 3 (0 min)→4 1 (40 min), linear gradient]; flow rate 1 0 ml min^{-1}, detection, UV_{203nm} (0 32 Aufs)

analysis of the diterpene glycosides [1]. In continuing the chemotaxonomical studies of this genus [2], 19α-hydroxyursane-type glycosides such as **1** and **2** were isolated from leaves of several *Rubus* spp.; *R. microphyllus* L.f., *R. koehneanus* Focke., *R. trifidus* Thunb. and *R. medius* O. Ktze. (tentatively named group B), etc. From leaves of these plants, no diterpene glycoside was isolated. The usefulness of HPLC analysis of glycosides for the chemical distinction of the source plants (group B) of Bog-bun-ja (= Fu-pen-zi) was conducted using the triterpene glycosides as marker substances. The HPLC analysis of glycoside-fraction of methanolic extracts from fruits of *R. coreanus* (Fig. 4) showed the occurrence of **1**–**4** and the absence of the diterpene-glycosides such as goshonosides. On the other hand, the HPLC of the extracts of fruits and leaves *R. crataegifolius* and *R. parvifolius* demonstrated the presence of **1**–**3** in the former and **1** and **2** in the latter, while no dimeric triterpene glycosides (**4**) and diterpene glycoside (goshonosides) were detected in fruits and leaves of either plant. The HPLC analysis of commercial Bog-bun-ja, from a Korean market, showed the very similar pattern to those of fruits of *R. coreanus*, being different from fruits of the other fruits of group B. Further, from this crude drug, **1**–**4** were isolated and identified. This indicates that this drug in the Korean market must be fruits of *R. coreanus*.

EXPERIMENTAL

General. Mps: uncorr. IR: KBr. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained at 40° in pyridine-d_5 or $CDCl_3$ using TMS as an int. standard. ^{1}H–^{1}H and ^{1}H–^{13}C COSY, NOEDS, ^{1}H–^{13}C LRCOSY and COLOC were taken by well-known methods EIMS were recorded at 70 eV. HPLC was carried out with a pump, CCPM (Tosoh Co. Ltd., Shin-Nanyou, Japan), and detection was accomplished with a differential refractometer and UV detector. The identification of each known compound was performed by comparison of the ^{1}H and ^{13}C NMR spectra, $[\alpha]_D$ and mp with those of an authentic sample.

Extraction and purification. Powder (500 g) of dried leaves of *Rubus coreanus*, which were cultivated at Toyama University, Japan, was extracted with MeOH. The extract (80 g) was suspended in H_2O and defatted with Et_2O. The H_2O layer was chromatographed on a column of Diaion HP-20 (Mitsubishi Kasei), by eluting successively with H_2O–MeOH, 1:0, 1:1, 1:4 and 0:1. The 80% MeOH eluate was chromatographed on silica gel (EtOAc–EtOH–H_2O, 16:2:1) to give 5 frs, I–V in order of elution. Fr. II (690 mg) was separated by HPLC [TSKgel ODS-120T, 30 cm × 21.5 mm, (Tosoh); MeOH–H_2O, 1:1] to give **1** (200 mg) and **2** (140 mg). Fr. IV (4.92 g) was applied to a column of silica gel (EtOAc–EtOH–H_2O, 12.2:1 and then 8 2.1), and finally purified by HPLC (TSKgel ODS-120T, 30 cm × 21.5 mm; MeOH–0.05% TFA, 52:48) to give **3** (1.85 g) and **4** (1.25 g).

In the same procedure as above, glycosides **1**–**4** were isolated from the leaves cultivated at Chung-Ang University, Korea, and also from commercial Bog-bun-ja purchased in Seoul: yields (%) from the Korean leaves; **1**, 0.018; **2**, 0.014; **3**, 0.37; **4**, 0.25: from the commercial bog-bun-ja; **1**, 0.12, **2**, 0.017, **3**, 0.21; **4**, 0.14.

Niga-ichigoside F1 (**1**). Needles (from MeOH–H_2O), mp 225–228°, $[\alpha]_D^{18}$ + 19.3° (MeOH; *c* 0.96). Niga-ichigoside F2 (**2**): needles (from MeOH–H_2O), mp 214–216°, $[\alpha]_D^{18}$ + 15.2° (MeOH; *c* 1.02). Suavissimoside R1 (**3**): needles (from MeOH), mp 285–288°, $[\alpha]_D^{18}$ + 20.6° (MeOH; *c* 0.67).

Coreanoside F1 (**4**). Needles (from MeOH–H_2O), mp 242–245° (decompd), $[\alpha]_D^{25}$ + 33.2° (MeOH; *c* 0.56). FABMS (positive) *m/z*: 1381 $[M+Na]^+$ 1219 $[M+Na-Glc]^+$, (negative) *m/z*: 1357 $[M-H]^-$, 1195 $[M-H-Glc]^-$, 679, 517 [679 −Glc]$^-$. HR-FABMS *m/z* 1381 7290, $C_{72}H_{110}O_{24}Na$ requires: *m/z* 1381.7291. IR ν_{max} cm^{-1} 3460 (–OH), 1726, 1712 (ester), 1686 (CO_2H) ^{1}H NMR (pyridine-d_5) δ6.28 and 6.32 (each 1H, each *d*, *J* = 7.8 Hz, H-1 of Glc and Glc'). ^{13}C NMR (sugar moieties): δ95.8 (Glc-1), 74.1 (Glc-2), 78.8 (Glc-3), 71.6 (Glc-4), 78.9 (Glc-5), 62.7 (Glc-6), 95.7 (Glc'-1), 74.1 (Glc'-2), 78.8 (Glc'-3), 71.5 (Glc'-4), 78.9 (Glc'-5), 62.7 (Glc'-6) ^{13}C NMR data of aglycone moiety is listed in Table 1.

Enzymic hydrolysis of **4**. Crude pectinase (Tanabe, 200 mg) was added to a soln of **4** (200 mg) in 50 mM acetate buffer (pH 5.6, 30 ml) and the mixture incubated for 4 days at 37°. The reaction mixture was extracted with *n*-BuOH (30 ml) D-Glc in the aq. layer was detected by GLC [9] The *n*-BuOH extract was deionized with Dowex 50W X8 (H^+ form) and chromatographed on silica gel ($CHCl_3$–MeOH, 5:1) to give compound **5** (100 mg).

Coreanogenoic acid (**5**). Powder, $[\alpha]_D^{24}$ + 5.7° (MeOH; *c* 0.51) (Found: C, 69.58, H, 8.90. $C_{60}H_{90}O_{14}$ requires C, 69.60; H, 8.76%) ^{1}H NMR (pyridine-d_5): δ1.01, 1.03, 1.11, 1.13, 1.35, 1.42, 1.57, 1.62, 1.78 (each 3H, each *s*, tert-Me × 9), 1.08, 1.09 (3H, each *d*, *J* = 6.7 Hz, H_3-30 and -30'), 2.97, 2.98 (each 1H, each *s*, H-19 and -19'), 4.03, 4.68 (1H, each *d*, *J* = 10.3 Hz, H_2-24), 4.16 (1H, *ddd*, *J* = 4.2, 10.5, 10.7 Hz, H-2), 4.28 (1H, *d*, *J* = 10.5 Hz, H-3), 5.15 (1H, *br d*, *J* = 10.7 Hz, H-2'), 5.46, 5.52 (1H, *t*-like, H-12 and -12'), 6.62 (1H, *br s*, H-3') ^{13}C NMR spectrum is listed in Table 1.

Methylation of compound **5**. A CH_2N_2–Et_2O soln (2 ml) was added to a soln of **5** (50 mg) in EtOH (0.5 ml) and the soln was left for 2 hr. Several drops of HOAc were added to the reaction mixture, and the soln evapd to dryness. The residue was purified by HPLC (TSKgel ODS-120T, 30 cm × 21.5 mm, MeOH–H_2O, 3.2) to give trimethyl ester of **5** (**6**)

Compound **6**. Powder, $[\alpha]_D^{24}$ + 2.4° (MeOH; *c* 0.98) (Found. C, 70.15; H, 9.02. $C_{63}H_{96}O_{14}$ requires: C, 70.23, H, 8.98%). IR-ν_{max} cm^{-1}: 3460 (–OH), 1734, 1726 (ester) FABMS (positive) *m/z*: 1099 $[M+Na]^+$, 1077 $[M+H]^+$. ^{1}H NMR: Table 2, ^{13}C NMR: Table 1

Alkaline hydrolysis of compound **5**. 2% K_2CO_3–EtOH (5 ml) was added to a soln of **5** (100 mg), and the soln refluxed for 4 hr. The reaction mixture was acidified to pH 3.5 with 2% HCl, and then extracted with EtOAc. The EtOAc extract was separated by HPLC (TSKgel ODS-120T, 30 cm × 21.5 mm, MeOH–0.05% TFA, 9:1) to give **7** (25 mg) and **8** (30 mg). Compound **7**: powder, $[\alpha]_D^{24}$ + 29.5° (MeOH, *c* 0.45). Compound **8**: powder, $[\alpha]_D^{21}$ + 45.5° (MeOH; *c* 0.98). ^{1}H NMR (pyridine-d_5): δ0.92, 1.04, 1.39, 1.57 (each 3H, each *s*, *tert*-Me), 1.05 (3H, *d*, *J* = 6.7 Hz, H_3-30), 2.81 (1H, *s*, H-18), 4.44, 4.65 (each 1H, each *d*, *J* = 10.3 Hz, H_2-23), 4.79 (1H, *br d*, *J* = 10.2 Hz, H-2), 5.18 (1H, *br s*, H-3), 5.46 (1H, *t*-like *s*, H-12) ^{13}C NMR: Table 1.

Methylation of compound **7** Compound **7** (15 mg) was treated with CH_2N_2–Et_2O soln to give **9** (15 mg). Compound **9**: needles from EtOAc–*n*-hexane, mp 103–104.5°, $[\alpha]_D^{21}$ + 30.2° (MeOH; *c* 0.87). ^{1}H NMR Table 2, ^{13}C NMR: Table 1

Methylation of compound **8**. Compound **8** (15 mg) was methylated with CH_2N_2 in the same way as above to give **10** (14 mg). Compound **10**: powder, $[\alpha]_D^{20}$ + 50.2° (MeOH; *c* 0.98. (Found: C, 68.01, H, 9.01. $C_{32}H_{50}O_8$ requires: C, 68.30; H, 8.96%) IR-ν_{max} cm^{-1}: 3460 (OH), 1727, 1719 (ester). FABMS (positive) *m/z*: 563 $[M+H]^+$, 545 $[M+H-H_2O]^+$, 527 $[M+H-2H_2O]^+$; (negative) *m/z*: 561 $[M-H]^-$, 547 $[M-Me]^-$, 529 $[M-Me-H_2O]^-$. ^{1}H NMR Table 2, ^{13}C NMR: Table 1.

Acetylation of compound **10**. Compound **10** (10 mg) was treated with Ac_2O–pyridine, 1:1 (1 ml) to give triacetate of **10** (**11**,

10 mg), which was subjected to ^{1}H NMR, ^{1}H–^{1}H COSY and NOEDS. Compound **11**. powder, $[\alpha]_D^{21}$ +38.0° ($CHCl_3$; *c* 0.5). EIMS *m/z*: 688 $[M]^+$, 670 $[M-H_2O]^+$, 628 $[M-HOAc]^+$ and $[M-HCO_2Me]^+$, 278. ^{1}H NMR: Table 2, ^{13}C NMR. Table 1.

Methylation of compound **4**. A MeOH soln (1 ml) of **4** (100 mg) was treated with CH_2N_2–Et_2O soln (5 ml) to give **12** (100 mg). Compound **12**: powder, $[\alpha]_D^{21}$ +31.5° (MeOH; *c* 1.05). IR-ν_{max} cm^{-1}. 3400 (OH), 1730, 1716 (ester). FABMS (positive) *m/z* 1395 $[M+Na]^+$, 1187 $[M-162]^+$ (negative) *m/z*· 1371 $[M-H]^-$, 1209 $[M-H-Glc]^-$, 1047 $[M-H-2Glc]^-$ HR-FABMS *m/z* 1395.7420, $C_{73}H_{112}O_{24}Na$ requires *m/z* 1395 7422. ^{1}H NMR (pyridine-d_5) δ0.83, 0 87, 0 97, 1.07, 1 27, 1 34, 1 58, 1.59 and 1 78 (each 3H, each *s*, *tert*-Me), 1 04 and 1 06 (each 3H, each *d*, *J* = 6 7 Hz, H_3-30 and -30′), 2.76 and 2.78 (each 1H, each *s*, H-19 and -19′), 3 58 (3H, *s*, 24-CO_2Me), 4 92 (1H, *br d*, *J* = 10 3 Hz, H-2), 5 46 and 5 53 (each 1H, each *t*-like, H-12 and -12′), 6 28 and 6.32 (each 1H, each *d*, *J* = 8 1 Hz, H-1 of Glc and Glc′), 6.58 (1H, *br s*, H-3). ^{13}C NMR (sugar moieties): δ95.8 (Glc-1), 74.2 (Glc-2), 78.7 (Glc-3), 71.7 (Glc-4), 78 9 (Glc-5), 62 6 (Glc-6), 95.7 (Glc′-1), 74 0 (Glc′-2), 78 7 (Glc′-3), 71 5 (Glc′-4), 78.8 (Glc′-5), 62.4 (Glc′-6) ^{13}C NMR data of aglycone moiety· Table 1.

Enzymic hydrolysis of compound **12**. Crude pectinase (Tanabe, 100 mg) was added to a soln of **12** (60 mg) in 50 mM acetate buffer (pH 5.6, 30 ml) and the mixture was incubated for 4 days at 37°. The reaction mixture was extracted with *n*-BuOH (30 ml), and the *n*-BuOH extract was deionized with Dowex 50W X8 (H^+ form) and chromatographed on silica gel ($CHCl_3$– MeOH, 10·1) to give **13** (40 mg) Compound **13**. powder, $[\alpha]_D^{21}$ + 3 7° (MeOH, *c* 1.05) (Found: C, 69.78, H, 8 90 $C_{61}H_{92}O_{14}$ requires C, 69.82, H, 8.94%). IR ν_{max} cm^{-1} 3400 (OH), 1730, 1716 (ester), 1680 (CO_2H). FABMS (positive) *m/z*. 1071 $[M+Na]^+$, 1053 $[M+Na-H_2O]^+$, 1049 $[M+H]^+$, 1035 $[M+Na-2H_2O]^+$, 1031 $[M+H-H_2O]^+$, (negative) *m/z* 1047 $[M-H]^-$, 1033 $[M-Me]^-$, 1029 $[M-H-H_2O]^-$, 1015 $[M-Me-H_2O]^-$, 1011 $[M-H-2H_2O]^-$, 997 $[M-Me-2H_2O]^-$ ^{1}H NMR (pyridine-d_5) δ0 81, 0 87, 0 95, 1.07, 1 29, 1 34, 1 57, 1 61 and 1.78 (each 3H, each *s*, tert-Me), 1.02 and 1.04 (each 3H, each *d*, *J* = 6.7 Hz, H_3-30 and -30′), 2 76 and 2 78 (each 1H, each *s*, H-19 and -19′), 3 58 (3H, *s*, 24-CO_2Me), 4 92 (1H, *br d*, *J* = 10.3 Hz, H-2), 5 46 and 5 53 (each 1H, each *t*-like, H-12 and -12′), 6 58 (1H, *br s*, H-3) The ^{13}C NMR spectrum is listed in Table 1

HPLC analysis of glycosides. Fruits or leaves of *Rubus* spp. (2 g) was extracted with MeOH A suspension of extract in H_2O (1 ml) was applied on a column of DIAION HP-20 (10 ml), washed with 50% MeOH (20 ml), and then eluted with 80% MeOH (20 ml) The 80% MeOH eluate was concd to 1 ml, applied on a YMC-Dispo C_{18} (YMC Co Ltd, Kyoto), washed with 50% MeOH (5 ml), and then eluted with 80% MeOH (5 ml) The 80% MeOH eluate was analysed by HPLC [TSKgel Amide-80, 25 cm × 4 0 mm, MeCN–0 05% TFA, 37 3 (0 min)→4 1 (40 min), linear gradient, flow rate, 1 0 ml min^{-1}; detection, $UV_{210\,nm}$ (0.64 aufs)]

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