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ENT-LABDANE-TYPE DITERPENE GLUCOSIDES FROM LEAVES OF *RUBUS CHINGII*

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Key Word Index—*Rubus chingii*; *Rubus suavissimus*; Rosaceae; glucosides of *ent*-labdane type diterpenes; goshonosides F1-5.

Abstract—Previously, a sweet steviol bisglucoside named rubusoside was isolated from leaves of a Chinese *Rubus* spp. which was tentatively assigned as *R. chingii*. From leaves of Japanese *Rubus chingii* (Japanese name Gosho-Ichigo) which are not sweet, five *ent*-labdane-type diterpene glucosides named goshonosidies F1-5 were isolated instead of rubusoside and their structures were elucidated. The name '*R. suavissimus*' has been proposed for the Chinese plant.

INTRODUCTION

As a part of our Chinese–Japanese cooperative studies on the sweet principles of Chinese plants [1–4], the sweet steviol-bisglucoside (**1**, rubusoside) was recently isolated in high yield (5.3 %) from leaves of a *Rubus* species which grows wild in the southern province of China and is used as a sweet tea [1]. This is the first example of the isolation of a diterpene-glycoside from Rosaceae. It was noted that **1** had already been obtained [5] from stevioside (**2**), the major sweet principle of *Stevia rebaudiana* (Compositae) [6] by partial enzymic hydrolysis and was used as an important intermediate for the chemical conversion of **2** into rebaudioside A (**3**) [5, 7] which is a better *Stevia*-sweetener than **2**. This rosaceous plant was tentatively designated as *Rubus chingii* Hu. A plant with the same name grows wild in Japan (Japanese name: Gosho-Ichigo), though its leaves do not taste sweet. This paper reports the isolation and structure determination of several diterpene glucosides from Japanese *R. chingii* Hu.

RESULTS AND DISCUSSION

Column chromatography of a glycoside fraction from the methanolic extract of the dried leaves collected in Japan afforded five new glycosides which we have named goshonosides-F1 (**4**), -F2 (**5**), -F3(**6**), -F4(**7**) and -F5(**8**) in yields of 5.7, 0.2, 0.2, 0.4 and 0.8 %, respectively. Mineral acid hydrolysis of these glycosides yielded glucose. Hydrolysis of **4**, **5** and **8** with crude hesperdinase [9] afforded a common aglycone, $C_{20}H_{34}O_3$ (**9**), while on the same treatment **6** and **7** yielded the aglycones, $C_{20}H_{32}O_3$ (**10**) and $C_{20}H_{34}O_2$ (**11**), respectively. Comparison of the ^{1}H and ^{13}C NMR signals with those of agatholal (**12**) [10] revealed the presence of the same allylalcohol side chain system as that of **12** in all of these aglycones (Tables 1 and 2). Besides these signals, the ^{1}H and ^{13}C NMR spectra of **11** exhibited signals due to one vinyl group, two quarternary methyl groups and one $-CH_2OH$ group attached to a quarternary carbon (Tables 1 and 2). The ^{13}C NMR of **11** further indicated the presence of seven methylenes, two methines and two quarternary carbons. Taking into account the biogenetical considerations, these data suggested that **11** was 15-hydroxy-labda-8(17),13-diene in which the 18-, 19- or 20-methyl group was oxidized to a primary alcohol. This was confirmed by comparison of the ^{13}C NMR spectrum of **11** with those of the labdane-type diterpene, methyl copaiferate (**13**) [11] (Table 2); signals due to C-1, C-2, C-6, C-7, C-8, C-9, C-10, C-11, C-17 and C-20 of **13** appeared at very similar positions to those in the spectrum of **11**, excluding the possibility of the presence of a hydroxy group at C-20. Further, signals assigned to C-1, C-2, C-3, C-4, C-5, C-18, C-19 and C-20 of the 18-hydroxyditerpene (**14**) [12] were observed at very similar positions in the spectrum of **11**, while those of the 19-hydroxyditerpene (**15**) [13, 14] could not be found at the corresponding positions of the spectrum of **11** (Table 2). The location of a hydroxyl group of **11** at C-18 (equatorial $-CH_2OH$) was also substantiated by the ^{1}H NMR; the carbinol proton signals of **11** (Table 1) appeared at almost the same positions as those of **14** (a pair of AB-doublets centred at δ 3.22, J(Hz) = 10.5, 100 MHz, $CDCl_3$) [12], being evidently different from those of **15** (a pair of AB-doublets centred at δ 3.60, J (Hz) = 11.0, 100 MHz, $CDCl_3$) [13].

The IR spectrum of **10** showed a band at 1720 cm^{-1} (Nujol) and in the ^{13}C NMR spectra (Table 2), on going from **11** to **10**, the signal due to the 18-carbinol carbon was replaced by a signal at δ 180.9 assignable to a carboxyl carbon, while other carbon signals appeared at almost the same positions as those of **11**. $LiAlH_4$ reduction of the methyl ester (**16**) of **10** afforded an alcohol which was identical with **11** including the optical rotation. The negative Cotton effect of the CD curve of **10** ($\Delta\varepsilon_{220}$ − 0.48 (MeOH; c 0.050)) indicated the *ent*-type absolute configuration [15]. It follows that **10** and **11** can be formulated as *ent*-labda-8(17),13-dien-15-ol-18-oic acid and *ent*-labda-8(17),13-diene-15,18-diol, respectively.

The anomeric proton resonances (Table 1) and carbon

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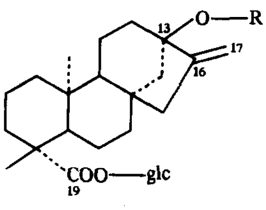
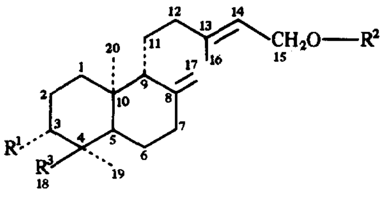


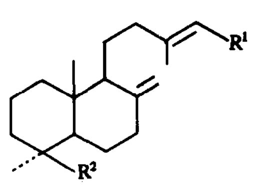
1 R = glc
2 R = glc^{2}—glc
3 R = glc$<^{2\text{—glc}}_{3\text{—glc}}$

glc : β-D-glucopyranosyl



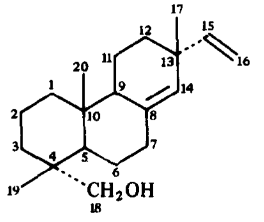
	R¹	R²	R³
4	OH	glc	CH_2OH
5	OH	H	CH_2O—glc
6	H	glc	COO—glc
7	H	glc	CH_2O—glc
8	OH	glc	CH_2O—glc
9	OH	H	CH_2OH
10	H	H	COOH
11	H	H	CH_2OH
16	H	H	COOMe
18	OAc	Ac	CH_2OAc
21	OH	Ac	CH_2OAc
22	OAc	Ac	CH_2OH

glc: β-D-glucopyranosyl



	R¹	R²
12	CH_2OH	CHO
13	COOMe	Me
17	CH_2O—xyl	CHO

xyl: β-D-xylopyranosyl



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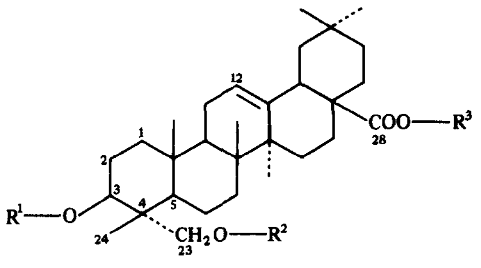
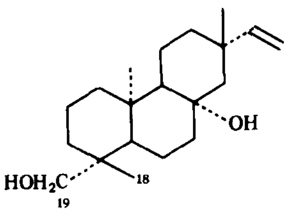


	R¹	R²	R³
19	H	H	H
20	Ac	Ac	Me
23	H	Ac	Me
24	H	H	Me
25	Ac	H	Me



15

signals due to sugar moieties (Table 2) indicated the presence of two β-glucopyranosyl units in **7**. On going from **11** to **7**, the carbon signals due to C-18 were displaced by +6.8 ppm [16] and those of C-13, C-14 and C-15 were displaced by +3.0, −4.9 and +6.8 ppm respectively as were observed for the β-xyloside (**17**) of **12** [10], while other carbon signals remained almost unchanged. Consequently, **7** can be formulated as the 15,18-di-*O*-β-glucopyranoside of **11**.

Two anomeric proton signals (Table 1) and the carbon signals due to sugar carbons (Table 2) showed the presence of two β-glucopyranosyl units in **6**, one of which must be an ester type based on its anomeric carbon chemical shift (at δ 96.0) [17]. The signals due to C-13, C-

Table 1. ^{1}H NMR data of compounds **4–12**, **18**, **21** and **22** [90 MHz (**18** at 270 MHz), TMS as int. standard]

Compound	H-3	H-14	H_2-15	H_3-16	H_2-17	H_2-18	H_3-19	H_3-20	15glc H-1	18glc H-1	acetyl-Me
11†	—	5.40 *t* (7.2)*	4.15 *d* (7.2)	1.68 *s*	4.53, 4.84 *s* (*br*)	3.10, 3.43 *d* (10.8)	0.75 *s*	0.73 *s*	—	—	
7‡	—	5.53 *t* (6.3)	4.15 *d* (6.3)	1.63 *s*	4.53, 4.89 *s* (*br*)	3.39, 3.69 *d* (6.3)	0.77 *s*	0.70 *s*	4.85 *d* (7.2)	4.72 *d* (6.2)	
10	—	5.39 *t* (7.2)	4.17 *d* (7.2)	1.68 *s*	4.55, 4.85 *s* (*br*)	—	1.16 *s*	0.73 *s*	—	—	
6‡	—	5.59 *t* (6.0)	4.46 *d* (6.0)	1.65 *s*	4.57, 4.87 *s* (*br*)	—	1.30 *s*	0.69 *s*	4.91 *d* (7.2)	6.28 *d* (7.2)	
9	3.65 *m*	5.37 *t* (6.0)	4.13 *d* (6.0)	1.66 *s*	4.53, 4.85 *s* (*br*)	3.36, 3.65 *d* (9.6)	0.82 *s*	0.73 *s*	—	—	
9‡	4.18 *m*	5.78 *t* (7.2)	4.47 *d* (7.2)	1.71 *s*	4.65, § *s* (*br*)	3.66, 4.13 *d* (10.0)	1.02 *s*	0.81 *s*	—	—	
12†	—	5.35 *t* (6.8)	4.11 *d* (6.8)	1.65 *s*	4.53, 4.86 *s*	1.02 *s*	9.70 *s*	0.57 *s*	—	—	
18†	4.81 *dd* (4.3, 11.5)	5.31 *t* (6.6)	4.58 *d* (6.6)	1.70 *s*	4.55, 4.87 *s* (*br*)	3.74, 3.79 *d* (11.5)	0.82 *s*	0.75 *s*	—	—	2.03, 2.06, 2.08
21†	3.45 *m*	5.32 *t* (7.2)	4.58 *d* (7.2)	1.71 *s*	4.54, 4.87 *s* (*br*)	3.76, 4.20 *d* (12.0)	0.83 *s*	0.86 *s*	—	—	2.04, 2.10
22†	§	5.31 *t* (7.2)	4.58 *d* (7.2)	1.69 *s*	4.53, 4.85 *s* (*br*)	2.77, 3.31 *d* (12.0)	0.74 *s*	0.67 *s*	—	—	2.04, 2.07
4‡	§	5.61 *t* (6.6)	§ (6.6)	1.67 *s*	4.53, 4.94 *s* (*br*)	3.68, 4.15 *d* (12.0)	1.03 *s*	0.82 *s*	4.93 *d* (7.2)	—	
5‡	§	5.77 *t* (6.6)	4.48 *d* (6.6)	1.70 *s*	4.63, 4.93 *s* (*br*)	3.52, § *d* (10.2)	0.94 *s*	0.77 *s*	—	4.83 *d* (7.2)	
8‡	§	5.59 *t* (6.0)	4.11 *d* (6.0)	1.64 *s*	4.58, 4.93 *s* (*br*)	3.53, § *d* (9.0)	0.94 *s*	0.76 *s*	4.90 *d* (7.0)	4.82 *d* (7.0)	

*Coupling constants are in Hz.

†Run in $CDCl_3$.

‡Run in C_5D_5N.

§Overlapped with other signals.

Table 2. ^{13}C NMR data of compounds **6**, **7** and **10**–**14** (25.15 MHz, TMS as int. standard)

C	**11***	**11**†	**12**†	**13**†	**14**†	**7***	**10***	**6***
1	38.9	38.5	38.4	39.0	38.6	38.5	38.3*	38.0
2	19.3	18.6	19.3	19.1	18.1	18.9	19.0	18.7
3	36.0	35.3	38.5	42.0	35.1	36.3	37.7	37.0
4	38.5	38.0	48.6	33.3	37.1	37.8	47.7	47.9
5	48.5	48.4	55.0	55.3	47.3	48.1	50.1	49.5
6	24.5‡	24.0‡	22.1	24.4	22.0	24.6‡	27.3	26.8
7	38.9	38.3	34.5	38.1	35.4	38.1	38.3	38.0
8	149.0	148.3	147.3	148.3	136.8	148.9	148.5	148.2
9	56.7	56.1	56.1	56.1	50.2	56.2	56.7	56.5
10	39.8	39.5	40.1	39.5	37.8	39.6	39.2	39.0
11	22.4‡	21.7‡	24.1	21.1	18.5	21.9‡	22.1	21.9
12	38.5	38.0	38.5	39.6	34.2	38.5	38.7‡	38.5
13	137.5	140.3	139.7	161.0	37.5	140.5	137.4	140.4
14	126.0	123.0	123.5	114.9	128.3	121.1	125.9	121.1
15	59.0	59.3	59.2	167.2	148.8	65.2	58.9	65.7
16	16.5	16.3	16.3	19.1	109.8	16.4	16.4	16.5
17	106.5	106.4	107.3	106.2	25.6	106.3	107.1	107.0
18	71.4	71.9	24.4	33.3	71.5	79.1	180.9	177.3
19	18.2	17.6	205.5	21.1	17.7	18.0	17.3	17.0
20	15.2	14.9	13.6	14.4	15.3	15.2	14.9	14.9
OMe				50.5				
15-G-1						103.0		103.2
2						74.7		74.8
3						78.0		78.1‡
4						71.4		71.4
5						78.0		78.1‡
6						62.6		62.6§
18-G-1						105.3		96.0
2						74.7		74.0
3						78.0		78.4‡
4						71.4		70.8
5						78.0		79.1‡
6						62.6		61.9§

*Run in C_5D_5N.

†Run in $CDCl_3$.

‡, §Values with the same sign may be interchanged in the vertical column.

14 and C-15 of **6** appeared at almost the same positions as those of **7** and on going from **10** to **6**, the C-18 signal was displaced upfield by 3.6 ppm [17]. Accordingly, **6** can be formulated as the 18-β-glucopyranosyl ester of the 15-*O*-β-glucopyranoside of **10**.

A carbon signal of **9** at 72.8 (*d*) (Table 3) as well as a proton signal at δ 4.81 [*dd*, *J* (Hz) = 4.3 and 11.5, Table 1] of the triacetate (**18**) of **9** disclosed the presence of an equatorial secondary hydroxyl group having two protons at its vicinal carbon. Comparison of the ^{13}C NMR spectrum of **9** with those of **11** and hederagenin (**19**) showed that all the signals of **11** except for C-2, C-3, C-4, C-18 and C-19 appeared at very similar positions in the spectrum of **9**, while signals due to C-1, C-2, C-3, C-4, C-5, C-23 and C-24 of **19** [18] were observed at very similar positions of the spectrum of **9** (Table 3). The 3(equatorial),18-dihydroxy system was also supported by comparison of the ^{1}H NMR (270 MHz, $CDCl_3$) spectrum of **18** with that of diacetyl-hederagenin (**20**) [14]; signals due to 3-CH–OAc (*vide supra*) and 18-CH_2–OAc of **18** (Table 1) were observed at very similar positions to those of **20** (3-CH–OAc at δ 4.78 [1H, *dd*, *J* (Hz) = 5.5 and 11.0] and 18-CH_2–OAc at δ 3.70 and 3.88 [a pair of AB-doublets, *J* (Hz) = 11.5], which were evidently different from those of 3β-, 19-hydroxy compounds [14, 19].

The absolute configuration of **9** was established as follows. On acetylation with Ac_2O and C_5H_5N for 15 min, **9** afforded the 15,18-diacetate **21** [IR $\nu_{\max}^{CCl_4}$ cm^{-1}: 3530 (OH) and 1735 (C=O)], the 3,15-diacetate **22** together with **18**. The position of an acetoxyl group of **21** and **22** was established from the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) by consideration of the acylation shifts. The chirality of C-3 of **21** was revealed as *R* by the modified Horeau's method [20] by comparison with the result for the 23-acetate **23** prepared from hederagenin methyl ester (**24**) in a similar manner. It follows that **9** can be formulated as 3α,15,18-trihydroxy-*ent*-labda-8(17),13-diene.

An anomeric proton signal (Table 1) as well as carbon signals due to a sugar moiety (Table 3) showed the presence of one β-glycopyranosyl unit both in **4** and **5**. The structures of these glucosides were established by ^{13}C NMR spectrometry by application of the glycosylation shift rule [16]. The chemical shifts of signals

Table 3. ^{13}C NMR data of compounds **4**, **5**, **8**, **9**, **21** and **22** (25.15 MHz, pyridine-d_5, TMS as int. standard)

C	9	19	21	22	4	5	8
1	38.3	38.9	38.1	38.2	38.4	38.1	38.0
2	28.3	27.6	24.1	28.0	28.3	28.1	27.9
3	72.8	73.7	74.7	71.0	72.8	71.8	71.5
4	43.3	42.9	42.4	42.5	43.3	43.4	43.2
5	47.6	48.8	46.3	47.6	47.5	46.8	46.9
6	24.3†	18.7	23.7†	24.2†	24.3†	24.3†	24.3†
7	37.3	33.0	36.6	37.1	37.3	37.1	37.0
8	148.8	39.8	148.3	148.1	148.6	148.8	148.6
9	56.4	48.2	55.9	56.3	56.1	56.4	56.1
10	39.6	37.3	39.1	39.5	39.6	39.4	39.3
11	22.4†	23.8	22.1†	22.1†	22.1†	22.4†	22.2†
12	38.7	122.7	38.4	38.5	38.6	38.8	38.6
13	137.4	145.0	142.6	142.4	140.6	137.6	140.5
14	125.9	42.2	119.1	119.1	121.2	125.5	120.9
15	58.9	28.4	61.3	61.3	65.6	58.9	65.7
16	16.4	23.8	16.4	16.4	16.5	16.4	16.5
17	106.6	46.7	106.8	106.9	106.6	106.5	106.8
18(23)*	67.3	68.2	63.9	66.4	67.3	74.4	74.6
19 (24)	12.9	13.1	13.5	12.6	12.9	12.8	12.7
20 (25)	15.2	16.9	15.1	15.2	15.2	15.2	15.1
Me–CO–			20.1 (× 2)	20.7 (× 2)			
Me–CO–			170.6 (× 2)	170.5 (× 2)			
15-G-1					103.3		103.1
2					75.2		74.6
3					78.5		78.0
4					71.7		71.5
5					78.5		78.0
6					62.7		62.6
18-G-1						105.5	105.1
2						74.8	74.6
3						78.2‡	78.0
4						71.8	71.5
5						78.4‡	78.0
6						62.8	62.6

*Carbon numbers in parentheses are for **19**.

†, ‡Value with the same sign may be reversed in the vertical column.

assignable as C-13, C-14 and C-15 of **4** were identical with those of **6** and **7** (Table 3), leading to the formulation of **4** as the 15-*O*-*β*-glucopyranoside of **9**. On going from **9** to **5**, the signal due to C-18 was displaced downfield by 7.1 ppm and that due to C-3 was shielded by 1.0 ppm, while other carbon signals remained almost unshifted. Accordingly, **5** can be formulated as the 18-*O*-*β*-glucopyranoside of **9**. An anomeric proton signal (Table 1) and the sugar carbon signals (Table 3) of **8** indicated the presence of two *β*-glucopyranosyl units. Comparison of the ^{13}C NMR spectrum of **8** with those of **4** and **5** (Table 3) established the structure of **8** as the 15,18-di-*O*-*β*-glucopyranoside of **9**.

In the leaves of the Japanese *R. chingii* used in the present study, no rubusoside (**1**) or its derivative was detected, while from leaves of the Chinese sweet plant which contains **1**, no labdane-type diterpene glycoside had been isolated. The name *R. chingii* Hu was originally assigned to a plant which is distributed in the middle-eastern province of China (Tiangsu, Zhejiang, Anhui and Fujian) and the specimen from this province has been unambiguously confirmed to be taxonomically identical with Japanese 'Gosho-Ichigo' by Migo [8]. The sweet plant growing in the southern province of China (Kwangchow and Kwangsi) seems now to be taxonomically different from *R. chingii*, for which the name, '*R. suavissimus* S. Lee' has recently been proposed by the Chinese taxonomist, Lee [21].

EXPERIMENTAL

General procedures. NMR: 25°, solvents see Tables 1–3, TMS as int. standard; ^{13}C NMR: 25.15 MHz; ^{1}H NMR: 100 MHz or 270 MHz. Mps (micro hot-stage) uncorr. For reverse phase CC, LiChroprep RP-8 (Merck) and Diaion HP-20 (Mitsubishi Chem. Ind.) were used.

Plant material. R. chingii was collected at Nodani, Yamaguchi-ken (October 8, 1980) and Sameura, Kohchi-ken (May 9, 1981) and no significant difference in the glycoside composition was observed between both the specimens. The specimens were unambiguously identified by Emeritus Prof. H. Hara, University Museum, University of Tokyo (address: 7-3-1, Hongo, Bunkyo-ku, Tokyo 113 Japan) and also by Dr. T. Yamanaka, Department of Botany, Faculty of Education, Kohchi University (address: 2-5-1, Akebono-cho, Kohchi 780 Japan). The specimens have been deposited in the Herbarium of the University Museum, University of Tokyo and the plant has been cultivated at the Experimental Station of Medicinal Plants, Hiroshima University School of Medicine.

Extraction and separation of glycosides. The dried leaves (1.19 kg) were extracted with hot MeOH and the MeOH extract taken to dryness. A suspension of the resulting extract in H_2O was washed with Et_2O and then extracted repeatedly with 1-BuOH satd with H_2O. The combined BuOH layers were taken to dryness, to give the crude glycoside fraction (162 g). A portion of the crude glycoside fraction (100 g) was chromatographed on silica gel and eluted with mixtures of EtOAc–EtOH–H_2O (80 : 8 : 1, 18 : 2 : 1 and then 8 : 2 : 1, all homogeneous). This yielded four fractions, I–IV, in increasing order of polarity.

Fraction I was subjected to reverse phase CC on Diaion (MeOH–H_2O, 1 : 1) to give a mixture of **4** and **5** which was acetylated with Ac_2O–C_5H_5N in the usual manner. The resulting acetate mixture was recrystallized from MeOH–H_2O to give the hexa-acetate (**24**) (2.8 g) of **4**. The mother liquor was subjected to chromatography on Diaion (MeOH–H_2O, 17 : 3) to give the hexa-acetate (**25**) (1.2 g) of **5**. Each acetate was saponified by standing with 2% KOH–MeOH at room temp. overnight followed by neutralization with Amberlite MB-3 to give the corresponding glucoside, **4** or **5**.

4: a white powder, $[\alpha]_D^{22}$ − 50.2° (MeOH; *c* 0.75). **24**: colourless prisms, mp 122–123° (from MeOH–H_2O) $[\alpha]_D^{24}$ − 51.9° ($CHCl_3$; *c* 1.0). (Found: C, 61.67; H, 7.43. $C_8H_{56}O_{14}$ requires: C, 61.94; H, 7.66%.) **5**: a white powder, $[\alpha]_D^{23}$ − 28.8° (MeOH; *c* 0.75). **25**: colourless needles, mp 152–153° (from MeOH–H_2O) $[\alpha]_D^{24}$ − 43.4° ($CHCl_3$; *c* 1.0). (Found: C, 61.61; H, 7.54. $C_{38}H_{56}O_{14}$ requires: C, 61.94; H, 7.66%).

Fraction II was chromatographed on Diaion (MeOH–H_2O, 7 : 3) and then on silica gel (EtOAc–EtOH–H_2O, 20 : 2 : 1 homogeneous) to give **6**: a white powder, $[\alpha]_D^{23}$ − 34.0° (MeOH; *c* 0.71) (Found: C, 58.95; H, 8.46. $C_{32}H_{52}O_{13} \cdot \frac{3}{2}H_2O$ requires: C, 57.21; H 8.25%.)

Fraction III was chromatographed on Diaion (MeOH–H_2O, 1 : 1) and then on silica gel (EtOAc–EtOH–H_2O, 20 : 2 : 1 homogeneous), affording **7**: a white powder, $[\alpha]_D^{16}$ − 37.7° (MeOH; *c* 0.96). (Found: C, 58.70; H, 8.49. $C_{32}H_{54}O_{12} . \frac{3}{2}H_2O$ requires: C, 58.43; H, 8.73%.)

Fraction IV was homogeneous, affording **8**: a white powder, $[\alpha]_D^{14}$ − 42.6° (MeOH; *c* 1.0). (Found: C, 56.12; H, 8.63. $C_{32}H_{54}O_{13} . 2H_2O$ requires: C, 56.29; H, 8.56%.)

Acid hydrolysis of **4–8**. A soln of each glucoside (3 mg) in 2.5% HCl–50% aq. dioxane (1 ml) was heated at 100° for 3 hr and the soln evaporated to dryness by blowing N_2 gas over it. The residue was heated with a few drops of trimethylsilylimidazole at 80° for 1 hr and then the reaction mixture was diluted with H_2O and extracted with hexane. TMSi-glucose was detected in the hexane layer by GC: detector: dual FID; carrier gas N_2 at 50 ml/min; column packed with 2% SE30 2 m × 3 mm, isothermal 180°; injection and detector temps: 230°. R_t of TMSi-glucose: 3.52, 4.35 and 6.85 min.

Enzymic hydrolysis of **4–8**. A soln of each glucoside (1.0 g) and crude hesperidinase (1.0 g; Tanabe Pharm. Ind Co. Ltd. Osaka, Japan) in McIlvain buffer (pH 4.0, 50 ml) was incubated at 37° overnight. The reaction mixture was extracted with $CHCl_3$ and the $CHCl_3$ layer was dried and taken to dryness. The residue was recrystallized to give each aglycone in an almost quantitative yield.

From **4**, **5** and **8**, **9** was obtained as colourless prisms, mp 141–142° (from $CHCl_3$), $[\alpha]_D^{15}$ − 28.3° ($CHCl_3$; *c* 1.4). (Found: C, 72.19; H, 10.92. $C_{20}H_{34}O_3 . \frac{1}{2}H_2O$ requires: C, 72.47; H, 10.64%.) EIMS 70 eV, *m/z* 322 $[M]^+$.

From **6**, **10** was obtained as colourless prisms, mp 141° (from $CHCl_3$), $[\alpha]_D^{16}$ − 30.0° ($CHCl_3$, *c* 0.67). (Found: C, 73.15; H, 9.90. $C_{20}H_{32}O_3 . \frac{1}{2}H_2O$ requires: C, 72.91; H, 10.10%.) EIMS 70 eV, *m/z* 320 $[M]^+$.

From 7, **11** was obtained as colourless prisms, mp 111–112° (from MeOH), $[\alpha]_D^{15}$ − 39.1° ($CHCl_3$; *c* 0.93). (Found: 78.37; H, 11.20. $C_{20}H_{34}O_2$ requires: C, 78.38; H, 11.18%.) EIMS 70 eV, *m/z* 306 $[M]^+$.

Complete acetylation of **9**. Acetylation of **9** with Ac_2O–C_5H_5N in the usual way afforded **18**, colourless needles, mp 133–134° (from MeOH–$CHCl_3$), $[\alpha]_D^{21}$ + 31.0° (MeOH; *c* 1.0). (Found: C, 69.40; H, 9.01. $C_{26}H_{40}O_6$ requires: C, 69.61; H, 8.99%.)

Reduction of **10** *to* **11**. To an Et_2O-soln of a methyl ester (38 mg) which was prepared from **10** with CH_2N_2 in Et_2O, was added $LiAlH_4$ (100 mg) and the mixture was stirred at room temp. for 1 hr. After adding a small amount of EtOAc to decompose excess reagent, the reaction mixture was washed with 10% HCl and H_2O, successively and evaporated to dryness. The residue was recrystallized from $CHCl_3$–MeOH to give **11** (18 mg) as colourless prisms, mp 111° $[\alpha]_D^{18}$ + 38.5° ($CHCl_3$; *c* 0.10), identification of which was confirmed by comparison of its ^1H and ^{13}C NMR spectra and other physical constants with those of an authentic sample.

Partial acetylation of **9**. A soln of **9** (500 mg) in a mixture of Ac_2O (10 ml) and C_5H_5N (10 ml) was allowed to stand at room temp. for 12 min and then the reaction mixture was poured into ice H_2O. The ppt was extracted with Et_2O and the Et_2O layer was evaporated to dryness. The residue was chromatographed on silica gel and eluted with C_6H_{14}–Et_2O (5 : 3 then 1 : 2) to give **21** (437 mg) and **22** (76 mg) together with a small amount of **18**. **21**: colourless oil, $[\alpha]_D^{21}$ − 38.8° (MeOH; *c* 1.0), high resolution EIMS 70 eV: $[M]^+$ Found: *m/z* 406.2681. $C_{24}H_{38}O_5$ requires 406.2716. **22**: colourless oil, $[\alpha]_D^{19}$ − 38.0° (MeOH; *c* 1.52), high resolution EIMS 70 eV: $[M]^+$ Found: 406.2697. $C_{24}H_{38}O_5$ requires 406.2716.

Partial acetylation of **24**. Mild acetylation of **24** (1.4 g) under the same conditions as that of **9** and CC of the product on silica gel ($CHCl_3$–EtOAc, 1 : 1) afforded **23** (1.0 g) and the 3-acetate **25** (5 mg). **23**: colourless prisms, mp 94–96° (from MeOH), $[\alpha]_D^{23}$ + 80.3° (MeOH; *c* 1.0), IR: $\nu_{max}^{CCl_4}$ cm^{-1}: 3530 (OH) and 1724 (ester). Found: C, 74.20; H, 9.62. $C_{33}H_{52}O_5 . \frac{1}{4}H_2O$ requires C, 74.33; H, 9.92%. **25**: a white powder, $[\alpha]_D^{22}$ + 89.7° (MeOH; *c* 1.0). Found: C, 74.53; H, 9.92. $C_{33}H_{52}O_5$ requires: C, 74.96; H, 9.91.

Modified Horeau's method for **21** *and* **23**. A soln of **21** or **23** (7 mg) and DL-2-phenylbutylic acid anhydride (12 μl) in dry C_5H_5N was allowed to stand in a sealed microtube at room temp. for 20 hr. To the reaction mixture, was added (+)-(*R*)-α-phenylethylamine (12 μl) and after 30 min, the mixture was concd to dryness by blowing N_2 gas over it. The residue was extracted with a small amount of EtOAc and the soln was subjected to GC analysis; condition: dual FID; carrier gas: N_2 1.2 kg/cm²; (a) column packed with 2% Poly I 1.5 m × 5 mm, isothermal 195°, injection temp: 200° detector temp: 220°. (b) column packed with 2% SE30 1.5 m × 5 mm, isothermal 195°, injection temp: 230°, detector temp: 220°. The relative proportions of the amides of (−)-(*R*)- and (+)-(*S*)-α-phenylbutylic acid were calculated by the areas of their respective peaks. Subtraction of the corresponding value from the reaction with cyclohexanol gave the increments of the percentage area representing the (−)-(*R*)-acid as follows; **21**: −14.7% at GC condition (a) and −15.4% at GC condition (b). **23**: +12.2% (a) and +10.2% (b).

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