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β-GLUCOSYL ESTERS OF 19α-HYDROXYURSOLIC ACID DERIVATIVES IN LEAVES OF *RUBUS* SPECIES

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Key Word Index—*Rubus microphyllus*; *R. koehneanus*; *R. trifidus*; *R. medius*; Rosaceae; triterpene glucosyl esters; 19α-hydroxyursolic acid derivatives.

Abstract—β-Glucosyl esters of A-ring oxygenated 19α-hydroxyursolic acids were isolated from the leaves of *Rubus microphyllus*, *R. koehneanus*, *R. trifidus* and *R. medius*. Comparisons of the glycoside fractions of the leaves of 39 *Rubus* species were conducted, indicating the chemotaxonomic significance of this type of glucosyl ester in this genus.

INTRODUCTION

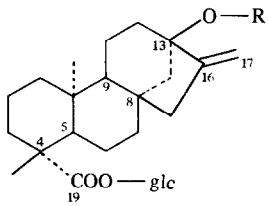
We have conducted co-operative studies on the chemical constituents of Chinese plants, isolating the sweet principle named rubusoside (**1**) in a high yield from leaves of the rosaceous plant collected in South China which was tentatively identified as *Rubus chingii* Hu in our first report [1]. However, the further taxonomic study of this plant led to the conclusion that it was not identical to *R. chingii* but must be named *R. suavissimus* S. Lee [2, 3]. This is the first example of the isolation of a diterpene-glycoside from rosaceous plants and it was noted that **1** was identical to the steviol-bisglucoside which had been prepared by the enzymatic partial hydrolysis of stevioside (**2**) [4], the major sweet principle of *Stevia rebaudiana* Bertoni (Compositae). In relation to this study, the chemical constituents of leaves of Japanese *R. chingii* which are not sweet were also investigated. This resulted in the isolation of several labdane-type diterpene glycosides named goshonosides F1–5 (**3**–**7**) in relatively high yields [3]. The present paper [5] deals with further studies on the glycoside composition of other *Rubus* species collected in Japan.

RESULTS AND DISCUSSION

A suspension of each methanolic extract of the dried leaves of 39 *Rubus* species collected in Japan (Table 1) was washed with diethyl ether and then extracted with 1-butanol saturated with water. The butanol extract was concentrated to dryness to give a crude glycoside fraction. Among these *Rubus* species, the TLC analysis of the glycoside fractions of 20 species including *R. suavissimus* and *R. chingii* indicated the presence of glycosides.

The yield of the glycoside fraction of leaves of *R. microphyllus* was relatively high (6.7%), so that this fraction was first subjected to chromatographic separation to give three new glucosides in yields of 0.5, 0.2 and 0.07%, respectively, which were named niga-ichigosides F1 (**8**), F2 (**9**) and F3 (**10**) in decreasing order of polarity on TLC.

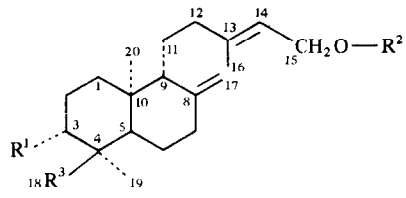
The aglycones of **8** and **9** were found to be unstable to either acid or alkaline treatment. Hydrolysis of **9** with crude hesperidinase yielded glucose and an aglycone (**11**). The electron-impact mass spectrum of a triacetate of **11** and comparison of the ^{13}C NMR spectrum of a methyl ester (**12**) of **11** with that of methyl clethroate (**13**) [6], as



1 R = glc

2 R = glc – glc

glc : β – D – glucopyranosyl



	R^1	R^2	R^3
3	OH	glc	CH_2OH
4	OH	H	CH_2O–glc
5	H	glc	COO–glc
6	H	glc	CH_2O–glc
7	OH	glc	CH_2O–glc

glc : β – D – glucopyranosyl

Table 1. TLC* comparison of the glycoside compositions of leaves of *Rubus* species

		Yield (%)†	1	3–7	8	9	10	17
R. suavissimus S. Lee	(—)‡	9.6	++	−	−	−	−	−
R. chingii Hu	(Gosho-Ichigo)	9.6	−	++	−	−	−	−
R. microphyllus L. f.	(Niga-Ichigo)	6.7	−	−	++	++	+	+
R. coreanus Miq.	(Tokkuri-Ichigo)	3.6	−	−	−	−	−	−§
R. illecebrosus Focke	(Bara-Ichigo)	8.4	−	−	++	−	±	±
R. pseudojaponicus Koidz.	(Himegoyō-Ichigo)	3.1	−	−	++	+	−	+
R. utchinensis Koidz.	(Hozaki-Ichigo)	4.4	−	−	+	±	−	+
R. corchorifolius L. f.	(Birōdo-Ichigo)	4.5	−	−	+	±	−	±
R. peltatus Maxim.	(Hasunoha-Ichigo)	4.2	−	−	−	−	−	−§
R. crataegifolius Bunge	(Kuma-Ichigo)	3.1	−	−	++	++	−	−
R. trifidus Thunb.	(Kaji-Ichigo)	2.2	−	−	+	++	−	++
R. hirsutus Thunb.	(Kusa-Ichigo)	3.7	−	−	±	±	−	−
R. nishimuranus Koidz.	(Nishimura-Ichigo)	2.5	−	−	+	+	−	++
R. sieboldii Bl.	(Hōroku-Ichigo)	5.9	−	−	++	−	−	−
R. medius O. Kuntze	(Himekaji-Ichigo)	3.7	−	−	+	+	−	++
R. minusculus Lévl. et Vant.	(Himebara-Ichigo)	5.4	−	−	−	±	−	±
R. babae Naruhashi	(Birōdokusa-Ichigo)	5.4	−	−	++	−	−	−
R. pseudoacer Makino	(Miyamamomiji-Ichigo)	4.8	−	−	++	−	−	−
R. kisoensis Nakai	(Kiso-Kiichigo)	3.9	−	−	−	−	−	−§
R. koehneanus Focke	(Miyamaniga-Ichigo)	9.7	−	−	++	++	−	+

No clear TLC spot due to glycosides was observed for the leaves of the following plants

R. vernus Focke	(Benibana-Ichigo)‡	*R. parvifolius* L.	(Nawashiro-Ichigo)
R. ikenoensis Lévl. et Vant.	(Goyō-Ichigo)	*R. lambertianus* Ser.	(Shimabara-Ichigo)
R. yenoshimanus Koidz.	(Togenashi-Kiichigo)	*R. ohmineanus* Koidz.	(Ōmine-Kiichigo)
R. palmatus Thunb.	(Momiji-Ichigo)	*R. pseudoyoshinoi* Naruhashi et Masaki	(Nagabanawashiro-Ichigo)
R. ohtakiensis Naruhashi	(Ōtaki-Kiichigo)	*R. pungens* Camb. E.	(Sanagi-Ichigo)
R. buergeri Miq.	(Fuyu-Ichigo)	*R. yoshinoi* Koidz.	(Kibinawashiro-Ichigo)
R. hiraseanus Makino	(Ōtokkuri-Ichigo)	*R. phoenicolasius* Maxim.	(Urajiro-Ichigo)
R. fruticosus L.	(Seiyōyabu-Ichigo)	*R. yabei* Lévl. et Vant.	(Miyamaurajiro-Ichigo)
R. hakonensis Fr. et Sav.	(Miyamafuyu-Ichigo)	*R. pedatus* J. E. Smith	(Kogane-Ichigo)
R. pectinellus Maxim.	(Marubafuyu-Ichigo)	*R. nesiotes* Focke	(Kuwanoha-Ichigo)

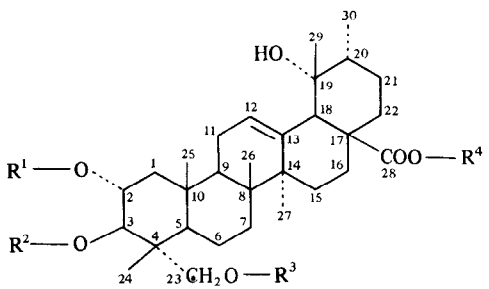
*On silica gel. Solvent: EtOAc–EtOH–H_2O (16:2:1, homogeneous) and $CHCl_3$–MeOH–H_2O (30:10:1, homogeneous).
†Yield of crude glycoside fraction.
‡Japanese name.
§The presence of other unidentified glycosides was demonstrated by TLC.

well as inspection of the ^1H NMR spectrum of the triacetate of **11** and comparison with the spectra of methyl tri-*O*-acetylclethroate (**14**) and 2,3-diacetoxytriterpenes, led to the formulation of **11** as 2α,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid. This compound had previously been isolated from *Coleus amboinicus* (Labiatae) [7]. The identification of **11** was confirmed by comparison of the ^1H NMR spectrum and TLC properties of **12** with those of an authentic specimen.

The ^{13}C NMR spectrum of **9** (Table 3) exhibited a set of signals attributable to an ester-type β-glucopyranosyl unit [8]. On going from **11** to **9**, it could be seen that the signal due to the 28-carboxyl carbon was displaced upfield by 3.6 ppm, while the other carbon resonances remained almost unshifted (Table 3). It follows that compound **9** can be formulated as the 28-β-glucopyranosyl ester of **11**. From the leaves of *R. koehneanus*, *R. trifidus* and *R. medius* (Table 1), compound **9** was also isolated in yields of 0.4, 0.15 and 0.02%, respectively.

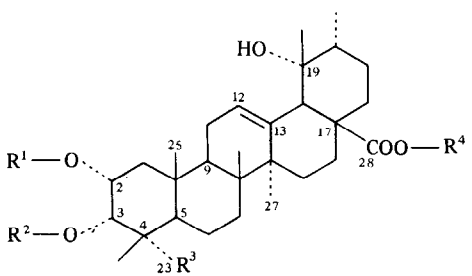
Hydrolysis of compound **8** with crude hesperidinase afforded glucose and an aglycone (**15**) which afforded the methyl ester triacetate (**16**). The electron-impact mass spectrum of **16** and comparison of the ^{13}C NMR spectrum of **15** with that of **11** (Table 2), as well as comparison of the ^1H NMR spectrum of **16** with that of methyl 2α,3β,23-triacetoxyolean-12-en-28-oic acid (= methyl tri-*O*-acetylarjunolate) [9], disclosed that the structure of **15** could be assigned as 2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid (= 19α-hydroxyasiatic acid), which had previously been isolated from *Symplocos spicata* (Symplocaceae) as a form of 3,28-bis-*O*-β-glucoside [10]. The identification of compound **15** was achieved by comparison of its TLC, ^1H NMR and ^{13}C NMR properties with those of an authentic sample.

In the ^{13}C NMR spectrum of **8** (Table 3), a set of signals due to an ester-type β-glucopyranoside unit [8] was identified and on going from **15** to **8**, it could be seen that the carbon signal assignable to the 28-carboxyl group was moved upfield by 3.9 ppm, while the other carbon signals remained at almost the same positions, leading to the assignment of the structure of **8** as the 28-β-glucopyranosyl ester of compound **15**. From the leaves of *R.*



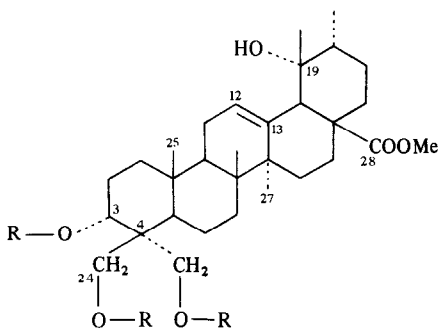
	R^1	R^2	R^3	R^4
8	H	H	H	glc
10	Ac	H	H	glc
15	H	H	H	H
16	Ac	Ac	Ac	Me

glc : β – D – glucopyranosyl



	R^1	R^2	R^3	R^4
9	H	H	CH_2OH	glc
11	H	H	CH_2OH	H
12	H	H	CH_2OH	Me
17	H	H	Me	glc
18	H	H	Me	H
19	Ac	Ac	Me	H

glc : β – D – glucopyranosyl



13 R = H

14 R = Ac

Table 2. ^{13}C NMR spectral data of compounds **11**, **15**, and **18** (25.15 MHz, pyridine, TMS as internal standard)

C	11	15	18
1	42.2	47.8	42.8
2	66.2	68.8	66.1
3	78.8	78.2	79.3
4	42.2	43.6	38.8
5	43.6	48.0	48.7
6	18.4	18.6	18.5
7	33.1	33.1	33.5
8	40.5	40.4	40.6
9	47.7	47.8	47.6
10	38.4	38.3	38.6
11	24.1	24.1	24.1
12	127.9	127.8	127.9
13	139.9	140.0	139.9
14	41.9	42.1	42.1
15	29.1	29.2	29.4
16	26.3*	26.3*	26.4*
17	48.2	48.2	48.2
18	54.6	54.6	54.6
19	72.7	72.6	72.6
20	42.2	42.3	42.3
21	26.9*	27.0*	27.0*
22	38.4	38.3	38.6
23	71.2	66.6	29.4
24	16.8	14.3	22.2
25	17.7†	17.3†	16.8†
26	17.3†	17.3†	17.3†
27	24.7	24.7	24.7
28	180.6	180.7	180.7
29	26.9	27.0	27.0
30	16.8†	16.8†	16.8†

*,† Values with the same symbol may be interchanged in the vertical column.

Table 3. ^{13}C NMR spectral data of compounds **8**–**10** and **17** (25.15 MHz, pyridine, TMS as internal standard)

C	8	9	10	17
1	47.9	42.1	44.6	42.8
2	68.8	66.3	74.0	66.0
3	78.2	78.8	74.0	79.0
4	43.5	42.1	44.2	38.6
5	48.5	43.5	47.7	48.4
6	18.7	18.5	18.6	18.5
7	33.1	33.1	33.0	33.4
8	40.6	40.7	40.6	40.7
9	47.9	47.8	47.5	47.6
10	38.3	38.4	38.4	38.6
11	24.2	24.2	24.2	24.1
12	128.3	128.3	128.1	128.2
13	139.2	139.3	139.3	139.1
14	42.1	41.9	42.1	42.0
15	29.1	29.1	29.1	29.3
16	26.0*	26.1*	26.1*	26.0*
17	48.5	48.6	48.6	48.4
18	54.4	54.4	54.4	54.3
19	72.5	72.6	72.6	72.5
20	42.1	42.6	42.1	42.0
21	27.0*	26.7*	26.9*	26.9*
22	37.7	38.4	37.7	37.6
23	66.6	71.3	65.6	29.0
24	14.2	16.7	14.3	22.2
25	17.5†	17.7†	17.4†	16.7†
26	17.5†	17.1†	17.4†	17.4†
27	24.5	24.6	24.6	24.5
28	176.8	177.0	176.9	176.8
29	27.0	26.7	26.9	26.7
30	16.7†	16.7†	16.7†	16.7†
28-G-1	95.7	95.8	95.7	95.6
2	73.9	74.0	74.0	73.8
3	78.8‡	78.8	79.1‡	78.7‡
4	71.2	71.3	71.3	71.1
5	79.0‡	78.8	78.9‡	79.0‡
6	62.3	62.3	62.4	62.3
OAc			21.4	
			170.8	

*,†,‡ Values with the same symbol may be interchanged in the vertical column.

koehneanus and *R. medius*, compound **8** was also isolated in yields of 0.5 and 0.06%, respectively, while in leaves of *R. trifidus*, compound **8** was detected only by TLC because of its low content (Table 1).

The presence of an acetoxyl group in a minor glycoside, compound **10**, was shown by its ^{13}C NMR spectrum (δ21.4 and 170.8), ^{1}H NMR spectrum (δ2.03, 3H, *s*) and FD mass spectrum (*m/z* 689 $[M + Na - MeCOOH]^+$). Although compound **10** could not be purified in a completely homogeneous state for elemental analysis because of its low content and the ready migration of its acetyl group, its structure was proposed to be the 2-acetate of compound **8** based on the acetylation shift [11] in its ^{13}C NMR spectrum (Table 3). In the ^{13}C NMR spectrum of the aglycone moiety of **10**, on going from **8** to **10**, it could be seen that the signal due to C-2 was displaced downfield by 5.2 ppm and those due to C-1 and C-3 were shielded by 3.3 and 4.2 ppm, respectively, while the other signals attributable to both sugar and aglycone moieties remained almost unshifted. This acetylated glycoside (**10**) was not detected in the leaves of *R. koeheanus*, *R. trifudus* or *R. medius*.

Besides these three glucosides, TLC of the glycoside fraction of leaves of *R. microphyllus* indicated the presence of two additional minor glycosides, one of which was found to be a major glycoside of the leaves of *R. trifidus*. Consequently, the glycoside fraction of leaves of *R. trifidus* was subjected to chromatography, affording a new glucoside, named kaji-ichigoside F1 (**17**), in a yield of 0.15% together with compound **9** (yield: 0.15%). The enzymatic hydrolysis of compound **17** yielded glucose and an aglycone (**18**). The electron impact mass spectrum and ^{1}H NMR spectrum of a diacetate (**19**) of compound **18**, as well as comparison of the ^{13}C NMR spectrum of **18** with those of **11** and **15** (Table 2), led to the formulation of compound **18** as 2α,3α,19α-trihydroxyurs-12-en-28-oic acid (= euscaphic acid), which had previously been isolated from *Euscaphis japonica* (Staphyleaceae) [12]. The identification of **18** was substantiated by comparison of the ^{1}H NMR spectrum of **19** with that of the reported data. The structure of compound **17** was assigned as the 28-*O*-β-glucopyranosyl ester of compound **18** by the ^{13}C NMR

spectrum in the same manner as compounds **8** and **9** (Table 3). From the glycoside fraction of leaves of *R. medius*, which is taxonomically very close to *R. trifidus*, compound **17** was also isolated as a major glycoside (yield: 0.16%), while **17** was detected only by TLC in the leaves of *R. microphyllus* and *R. koehneanus* because of its low content.

The TLC comparison of the glycoside compositions of the leaves of 20 *Rubus* species which showed the obvious glycoside spots is summarized in Table 1. The glycoside fraction of any plant in the present study did not exhibit the spots due to the diterpene glycosides **1** and **3–7**. This suggested the special chemotaxonomic position of both *R. suavissimus* and *R. chingii* in this genus. The presence of compounds **8**, **9** and (or) **17** was observed in the leaves of 15 species, indicating that the glucosyl ester of 19α-hydroxyursolic acid derivatives are rather common in this genus of Rosaceae, though the leaves of *R. coleanus*, *R. peltatus* and *R. kisoensis* were found not to contain **8**, **9** and **17**, while the presence of unidentified glycosides in these leaves was demonstrated by TLC.

It is notable that from *Sanguisorbia officinalis* (Rosaceae), the glycosides of pomolic acid (= 19α-hydroxyursolic acid) named ziyu-glycosides I and II were previously isolated [13] and recently, glucosyl esters of this type were also isolated from other rosaceous plants. The 28-β-glucosyl ester of tormentic acid (= 2α,19α-dihydroxyursolic acid) was obtained from *Rosa multiflora* [14] and compound **8** and the 28-β-glucosyl ester of 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid were isolated from the roots of *R. suavissimus* [15] and also from *Geum japonicum* [16].

Yamazaki and co-workers [17] reported the anti-gastric-stress-ulcer activity of A-ring polyhydroxyursolic acid derivatives. This pharmacological activity was demonstrated to be strong for the aglycones **11** and **15**, but to be very weak for **18** and the glucosides **8**, **9** and **17** [17].

EXPERIMENTAL

General procedure. NMR spectra were taken at 25° using TMS as internal standard; ^{13}C NMR at 25.15 MHz and ^1H NMR at 90 or 270 MHz. EIMS were taken at 75 eV ionization voltage. Mps were taken on a micro hot-stage and are uncorr. TLC: on silica gel (Kieselgel 60 F_{254} precoated, Merck) or on silanized silica gel (RP-8, precoated, Merck), detection, H_2SO_4. Acid hydrolysis of each glucoside and the identification of the resulting glucose by GC and TLC was carried out according to a previous paper [3].

Plant materials. Each plant material, collected in Japan, was botanically identified by one of the authors, N. Naruhashi; the specimens have been deposited at the Herbarium of Department of Botany, Faculty of Science, Toyama University.

Preparation of the glycoside fraction for preliminary comparison. Dried leaves (20 g) were extracted with hot MeOH and the MeOH extract was taken to dryness. A suspension of the resulting extract in H_2O was washed with Et_2O and then extracted with *n*-BuOH saturated with H_2O. The BuOH layer was taken to dryness to give the crude glycoside fraction. The comparison of this fraction from each plant is summarized in Table 1.

Extraction and separation of glycosides from the leaves of R. microphyllus. The dried leaves (680 g), collected in Gifu-shi, were extracted with hot MeOH and the MeOH extract was taken to dryness. A suspension of the resulting residue in H_2O was washed with Et_2O and then extracted with *n*-BuOH saturated with H_2O. The BuOH layer was taken to dryness to give the crude glycoside fraction (yield: 9.1%), which was chromatographed on highly porous polymer (MCI-gel HP-20, Mitsubishi Chem. Ind. Co. Ltd., Tokyo) and eluted with 60% MeOH. This yielded three fractions, I–III, in order of elution.

Fraction I was further chromatographed on highly porous polymer (MCI-gel CHP-20P, Mitsubishi Chem. Ind. Co. Ltd., Tokyo) (60% MeOH) and then on silanized silica gel (LiChroprep RP-8, Merck) (60% MeOH) to afford **8** in a yield of 0.5%.

Compound **8**: colourless needles, mp 231–232° (from MeOH–H_2O), $[\alpha]_D^{26}$ +11.2° (MeOH; *c* 1.01). (Found: C, 60.47; H, 8.63. $C_{36}H_{58}O_{11} \cdot 2\frac{1}{2} H_2O$ requires: C, 60.73; H, 8.91%.) EIMS (as heptaacetate) *m/z*: 960 $[M]^+$, 331 $[glc(Ac)_4]^+$; ^1H NMR (90 MHz, pyridine-d_5): δ6.23 (1H, *d*, *J* = 7.2 Hz, anomeric H). Mineral acid hydrolysis of **8** afforded glucose.

Fraction II was chromatographed on silica gel (EtOAc–EtOH–H_2O, 16:2:1, homogeneous) and then on LiChroprep RP-8 (60% MeOH) to yield **9** in a yield of 0.2%.

Compound **9**: colourless needles, mp 214–216° (from MeOH–H_2O), $[\alpha]_D^{20}$ +13.2° (MeOH; *c* 0.92). (Found: C, 62.78; H, 8.62. $C_{36}H_{58}O_{11} \cdot 1\frac{1}{2} H_2O$ requires: C, 62.81; H, 8.84%.) EIMS (as heptaacetate), *m/z*: 960 $[M]^+$, 331 $[glc(Ac)_4]^+$. ^1H NMR (90 MHz, pyridine-d_5): δ6.42 (1H, *d*, *J* = 6.9 Hz, anomeric H). On mineral acid hydrolysis, **9** yielded glucose.

Chromatography of fraction III on silica gel (EtOAc–EtOH–H_2O, 16:2:1, homogeneous) and then on LiChroprep RP-8 (60% MeOH) followed by HPLC on silanized silica gel (TSK gel LS-410, 7.5 mm i.d. × 60 cm); 70% MeOH; flow rate, 1.1 ml/min; detection, refractive index at room temp.) gave **10** in a yield of 0.07%, which was rather unstable and could not be obtained in a completely pure state.

Compound **10**: a white powder, FDMS *m/z*: 731 $[M+Na]^+$, 689 $[M+Na-HOAc]^+$, 569 $[M+Na-glc]^+$. ^1H NMR (90 MHz, pyridine-d_5): δ2.03 (3H, *s*, OCOMe), 6.30 (1H, *d*, *J* = 6.6 Hz, anomeric H). From leaves of the same plant collected in Hiroshima, **8** and **9** were isolated in similar yields to the above but **10** was not present in this leaves.

Enzymatic hydrolysis of compound **8**. A mixture of **8** (1.9 g) and crude hesperidinase [18] (1.9 g, Tanabe Pharm. Ind. Co. Ltd., Japan) in McIlvaine buffer (pH 4.0, 300 ml) was incubated at 37° for 10 hr. The mixture was extracted with EtOAc–*n*-BuOH (2:1) and the organic layer was chromatographed on silica gel ($CHCl_3$–MeOH, 10:1) to give **15** (580 mg). In the H_2O layer, glucose was detected by TLC.

Compound **15**: colourless micro-crystals, mp 283–285° (from MeOH), $[\alpha]_D^{17}$ +22.5° (MeOH; *c* 1.19). TLC: R_f 0.6 on silica gel (EtOAc–EtOH–H_2O, 32:2:1, homogeneous) and 0.3 on silanized silica gel (70% MeOH). This was identified by comparison of mp, $[\alpha]_D$ and ^{13}C NMR spectrum with an authentic sample obtained by Higuchi *et al.* [10]. Methylation of **15** with CH_2N_2 in Et_2O followed by acetylation with Ac_2O–pyridine afforded **16**: a white powder, $[\alpha]_D^{16}$ +24.7° ($CHCl_3$; *c* 1.75). EIMS *m/z*: 644 $[M]^+$, 584, 278, 260, 219, 201. ^1H NMR (270 MHz, $CDCl_3$): δ0.69, 0.89, 1.09, 1.21, 1.25 (each 3H, *s*, H-26, H-24, H-25, H-27, H-29, respectively); 0.95 (3H, *d*, *J* = 6.6 Hz, H-30); 1.92, 2.02, 2.09 (each 3H, *s*, OAc); 2.60 (1H, *s*, H-18); 3.59, 3.85 (each 1H, *d*, *J* = 11.9 Hz, H-23); 3.60 (3H, *s*, –COOMe); 5.08 (1H, *d*, *J* = 10.5 Hz, H-3); 5.17 (1H, *ddd*, *J* = 9.9, 9.9, 4.6 Hz, H-2); 5.35 (1H, *t*, *J* = 3.8 Hz, H-12).

Enzymatic hydrolysis of compound **9**. Compound **9** (470 mg) was hydrolysed with crude hesperidinase (650 mg) in the same way as **8** to give **11** (290 mg) and glucose.

Compound **11**: a white powder, $[\alpha]_D^{17}$ +25.3° (MeOH; *c* 1.05). Triacetate of **11**: a white powder, EIMS *m/z*: 630 $[M]^+$, 584, 264, 246, 219, 201. ^1H NMR (270 MHz, $CDCl_3$): δ0.74, 1.08, 1.10, 1.20, 1.31 (each 3H, *s*, H-26, H-24 (or H-25), H-25 (or H-24), H-27,

H-29, respectively); 0.96 (3H, *d*, J = 6.9 Hz, H-30); 1.95, 1.99, 2.05 (each 3H, *s*, OAc); 2.60 (1H, *s*, H-18); 3.74, 4.08 (each 1H, *d*, J = 10.5 Hz, H-23); 5.18 (1H, *d*, J = 3.1 Hz, H-3); 5.23 (1H, *ddd*, J = 12.3, 3.4, 3.4 Hz, H-2); 5.34 (1H, *t*, J = 3.1 Hz, H-12). Methyl ester (**12**): a white powder, $[\alpha]_D^{16}$ +31.9° ($CHCl_3$; *c* 1.65). ^{1}H NMR (90 MHz, $CDCl_3$): δ0.69, 0.74, 1.23, 1.29 (each 3H, *s*); 0.99 (6H, *s*); 2.60 (1H, *s*, H-18); 3.61 (3H, *s*, COOMe); 3.90 (1H, *m*, H-2); 5.38 (1H, *m*, H-12). Identified with an authentic sample [7] by comparison of ^{1}H NMR and TLC: R_f 0.3 on silica gel ($CHCl_3$–MeOH, 10:1) and 0.5 on silanized silica gel (70% MeOH).

Extraction and separation of glucosides from the leaves of R. koehneanus. The dried leaves (100 g), collected in Gifu, were extracted with hot MeOH and the resulting MeOH extract was separated in the same way as above to give a crude glycoside fraction (yield: 9.7%), which was chromatographed on silica gel (EtOAc–EtOH–H_2O, 32:2:1, homogeneous), yielding fractions I and II. Chromatography of fraction I on MCI gel CHP-20P (70% MeOH) and then on LiChroprep RP-8 (60% MeOH) gave **9** (yield: 0.4%), which was identified by comparison of mp, $[\alpha]_D$, ^{13}C NMR spectra and TLC (see Table 1) with those of an authentic sample.

Fraction II was subjected to chromatography on MCI gel CHP-20P (70% MeOH) to afford **8** (yield 0.5%) which was identified in the same way as above.

Extraction and separation of glucosides from the leaves of R. trifidus. A MeOH extract of the dried leaves (200 g), collected in Hiroshima, was separated in the same way as above to give a crude glycoside fraction (yield: 5.5%), which was separated into fractions I and II by chromatography on silica gel (EtOAc–EtOH–H_2O, 32:2:1, homogeneous). Chromatography of fraction I on LiChroprep RP-8 (70% MeOH) and then on silica gel ($CHCl_3$–MeOH, 5:1) gave **17**. Fraction II was chromatographed on MCI gel CHP-20P (60% MeOH) and on LiChroprep RP-8 (60% MeOH) successively to afford **9** (yield: 0.15%), which was identified by comparison of $[\alpha]_D$, ^{13}C NMR spectrum and TLC (see Table 1) with an authentic sample.

Compound **17**: a white powder, $[\alpha]_D^{16}$ +7.8° (MeOH; *c* 0.85). (Found: C, 62.65; H, 8.87. $C_{36}H_{58}O_{10} \cdot 2H_2O$ requires: C, 62.95; H, 9.10%.) EIMS (as hexaacetate) *m/z*: 331 $[(glc(Ac)_4)]^+$. ^{1}H NMR (90 MHz, pyridine-d_5): δ6.22 (1H, *d*, J = 7.0 Hz, anomeric H).

Enzymatic hydrolysis of compound **17**. Compound **17** (300 mg) was hydrolysed with crude hesperidinase in the same way as mentioned above to give **18** (50 mg).

Compound **18**: colourless micro-crystals, mp 272–273° (from MeOH) (lit. [12] 270–271°), $[\alpha]_D^{14}$ +20.4° (MeOH; *c* 1.59). Diacetate (**19**): colourless prisms, mp 189–190° (from C_6H_6–*n*-C_6H_{14}). EIMS *m/z*: 572 $[M]^+$, 526, 264, 246, 219, 201. ^{1}H NMR (90 MHz, $CDCl_3$): δ0.73, 0.87, 1.03, 1.20, 1.30 (each 3H, *s*, H-26, H-23, H-25 (or H-24 or H-30), H-27, H-29, respectively); 0.97 (6H, *s*, H-24, H-30 (or H-25)); 1.98, 2.13 (each 3H, *s*, OAc); 2.54 (1H, *s*, H-18); 4.95 (1H, *d*, J = 2.5 Hz, H-3); 5.17 (1H, *m*, $W_{1/2}$ = 11 Hz, H-2); 5.33 (1H, *m*, H-12). Identification of **19** was provided by comparison of mp, and ^{1}H NMR data with reported data [12].

Extraction and separation of glucosides of the leaves of R. medius. The MeOH extract of the dried leaves (200 g), collected at the Botanical Garden of Troyama University, was separated as described above to give a glycoside fraction (yield: 5.7%), which was chromatographed on silica gel (EtOAc–EtOH–H_2O, 16:2:1, homogeneous) to give fractions I and II.

Chromatography of fraction I on LiChroprep RP-8 (65% MeOH) and then on silica gel ($CHCl_3$–MeOH–H_2O, 80:10:1, homogeneous) gave **17** (yield: 0.16%), which was identified by comparison of TLC (see Table 1), $[\alpha]_D$ and ^{13}C NMR spectrum with those of an authentic sample. Fraction II, after chromatography on LiChroprep RP-8 (60% MeOH), gave **8** and **9** in yields of 0.06 and 0.02%, respectively. The identifications of both compounds were confirmed by comparison of TLC (see Table 1), $[\alpha]_D$ and ^{13}C NMR spectra with those of corresponding authentic specimens.

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