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Medicinal Foodstuffs. X.[1] Structures of New Triterpene Glycosides, Gymnemosides-c, -d, -e, and -f, from the Leaves of *Gymnema sylvestre* R. Br.: Influence of Gymnema Glycosides on Glucose Uptake in Rat Small Intestinal Fragments

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Following the characterization of gymnemosides-a and -b, new triterpene glycosides, gymnemosides-c, -d, -e, and -f, were isolated from the leaves of *Gymnema* (*G.*) *sylvestre* R. Br. Their chemical structures were elucidated on the basis of chemical and physicochemical evidence as follows: 21-*O*-benzoyl-28-*O*-acetylgymnemagenin 3-*O*-β-D-glucopyranosiduronic acid (gymnemoside-c), 23-*O*-[β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] gymnestrogenin (gymnemoside-d), 23-*O*-[β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 23-hydroxylongispinogenin (gymnemoside-e), 23-*O*-[β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 3β,16β,23,28-tetrahydroxyolean-18-ene (gymnemoside-f). The inhibitory effects of gymnemosides-c, -d, -e, and -f and principal triterpene glycosides from *G. sylvestre* on glucose uptake in rat small intestinal fragments were examined, and gymnemic acids II, III, and IV, gymnemasaponin V, and gymnemoside-f were found to exhibit the inhibitory activity.

Key words gymnemoside; *Gymnema sylvestre*; glucose uptake inhibitor; bioactive saponin; gymnemic acid; medicinal foodstuff

During the course of our studies in search of bioactive constituents of medicinal foodstuffs,[1,2] we have examined the inhibitory activity of the glycosidic fraction, which is commonly called "gymnemic acid," from the leaves of *Gymnema sylvestre* R. Br. (Asclepiadaceae), on the increase of serum glucose levels in oral glucose-loaded rats; "gymnemic acid" was found to show little activity. In order to evaluate the inhibitory activity of "gymnemic acid" in detail, we have isolated six new triterpene glycosides called gymnemosides-a (**5**), -b (**6**), -c (**1**), -d (**2**), -e (**3**), and -f (**4**), together with nine known triterpene glycosides, gymnemic acids I (**7**), II (**8**), III (**9**), IV (**10**), V, and VII and gymnemasaponins II, IV, and V (**12**) from "gymnemic acid". In the previous paper,[1] we reported the effect of "gymnemic acid" and the principal triterpene glycosides on the increase of serum glucose levels in oral glucose-loaded rats and the structures of gymnemosides-a (**5**) and -b (**6**). As a continuing study, we describe here the structural elucidation of the remaining new triterpene glycosides, gymnemosides-c (**1**), -d (**2**), -e (**3**), and -f (**4**), and the influence of the new gymnemosides and principal triterpene glycosides from "gymnemic acid" on glucose uptake in rat small intestinal fragments.[3]

Structures of Gymnemosides-c (1), -d (2), -e (3), and -f (4) Gymnemoside-c (**1**) was isolated as colorless fine crystals of mp 211.5—213.0 °C from aqueous methanol. The IR spectrum of **1** showed absorption bands at 3445, 1718, 1649, and 1044 cm^{-1} ascribable to hydroxyl, carboxyl, and ester functions, while its UV spectrum showed an absorption maximum at 228 nm (log ε 4.3) due to the benzoyl group. The molecular formula $C_{45}H_{64}O_{14}$ was determined from the negative-ion and positive-ion FAB-MS and by high-resolution MS measurement. Namely, a quasimolecular ion peak was observed at *m/z* 827 $(M-H)^-$ in the negative-ion FAB-MS of **1**, while its positive-ion FAB-MS showed quasimolecular ion peaks at *m/z* 829 $(M+H)^+$ and 851 $(M+Na)^+$. Alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide–50% aqueous dioxane (1 : 1, v/v) liberated gymnemagenin 3-*O*-glucuronide (**11**)[4] together with acetic acid and benzoic acid. The acetic acid was derived to the *p*-nitrobenzyl ester, which was identified by HPLC analysis.[5] Acid hydrolysis of **1** with 5% aqueous sulfuric acid furnished D-glucuronic acid, which was identified by gas-liquid chromatography (GLC) analysis of the trimethylsilyl thiazolidine derivative.[6] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by means of various NMR experiments,[7] showed signals assignable to an acetyl [δ 2.07 (s)], a benzoyl [δ 7.43 (dd, *J*=6.9, 7.3 Hz, 3‴-, 5‴-H), 7.53 (d, *J*=7.3 Hz, 4‴-H), 8.30 (d, *J*=6.9 Hz, 2‴-, 6‴-H)] and a gymnemagenin 3-*O*-glucuronide moiety [δ 3.73, 4.38 (both d, *J*=10.6 Hz, 23-H_2), 4.28 (m, 3-H), 4.67 (d, *J*=10.9 Hz, 22-H), 4.71, 5.10 (both d, *J*=10.9 Hz, 28-H_2), 5.19 (dd-like, 16-H), 5.28 (d, *J*=7.6 Hz, 1′-H), 5.43 (br s, 12-H), 5.96 (d, *J*=10.9 Hz, 21-H)]. Comparison of the ^{1}H-NMR and ^{13}C-NMR data for **1** with those for **11** indicated acylation shifts at the 21- and 28-positions. The positions of two acyl groups in **1** were clarified by a heteronuclear multiple bond correlation (HMBC) experiment, which showed long-range correlations between the 21-proton and the carbonyl carbon of the benzoyl group and between the 28-protons and the acetyl carbonyl carbon. On the basis of the above evidence, the structure of gymnemoside-c was determined to be 21-*O*-benzoyl-28-*O*-acetylgymnemagenin 3-*O*-β-D-glucopyranosiduronic acid (**1**).

Gymnemoside-d (**2**) was also isolated as colorless fine crystals of mp 219.1—221.0 °C from aqueous methanol. The IR spectrum of **2** showed strong broad absorption bands at 3410 and 1044 cm^{-1}, suggestive of an oligoglycosidic structure. The negative-ion and positive-ion FAB-MS of **2** showed quasimolecular ion peaks at *m/z* 945

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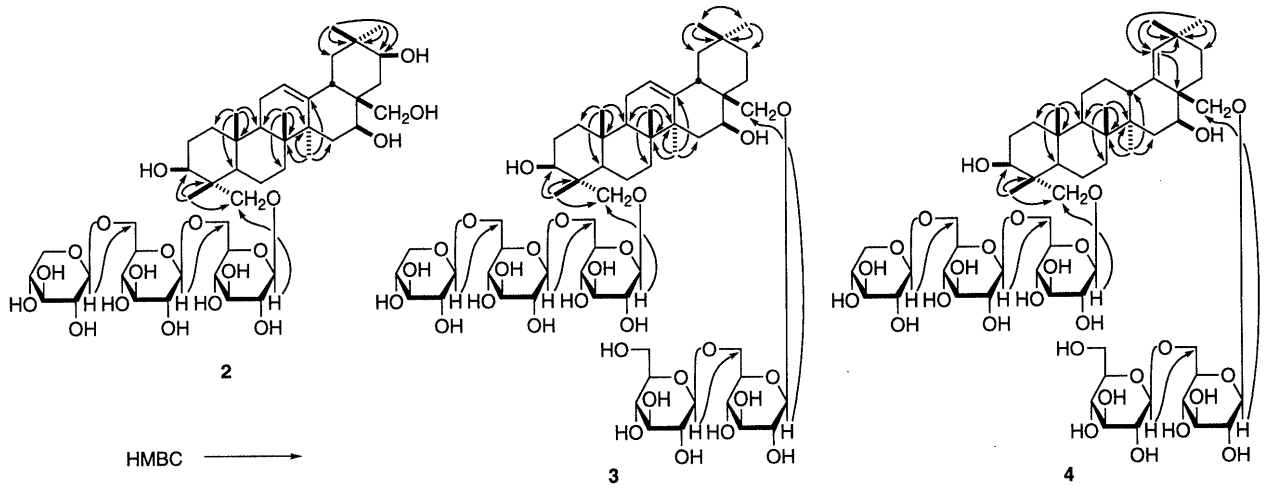


Fig. 1. HMBC Correlations of Gymnemosides-d (**2**), -e (**3**), and -f (**4**)

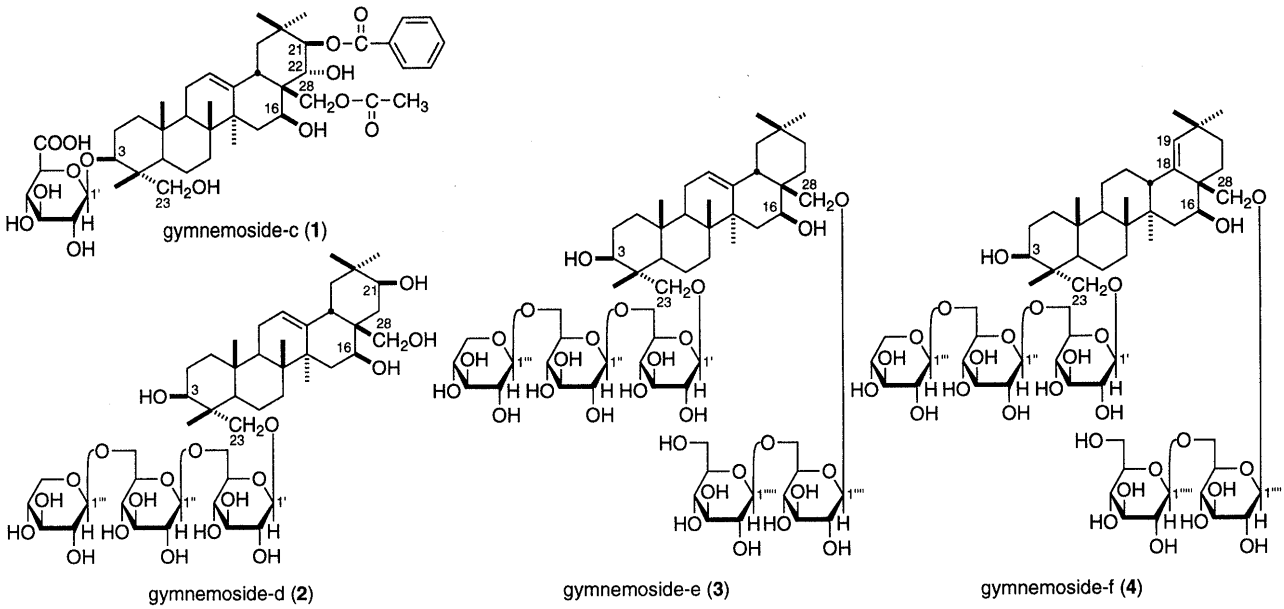
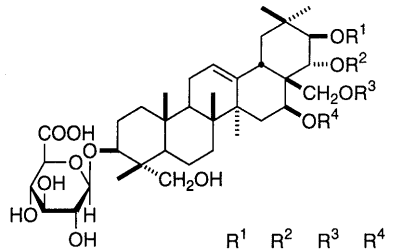


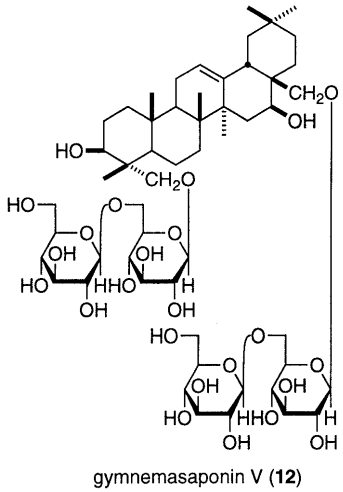
Chart 1

$(M-H)^-$ and 969 $(M+Na)^+$, respectively, and the molecular formula $C_{47}H_{78}O_{19}$ was determined by high-resolution MS measurement. Acid hydrolysis of **2** liberated gymnestrogenin (**13**),[8] D-glucose, and D-xylose. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1)[7] spectra of **2** indicated the presence of two β-D-glucopyranosyl moieties [δ 4.83 (d, J=7.6 Hz, 1′-H), 5.00 (d, J=7.6 Hz, 1″-H)] and a β-D-xylopyranosyl moiety [δ 4.92 (d, J=7.3 Hz, 1‴-H)] together with the gymnestrogenin part [δ 3.72, 4.48 (both d, J=10.6 Hz, 28-H_2), 3.97 (m, 23-H_2), 4.11 (m, 21-H), 4.18 (m, 3-H), 4.67 (dd-like, 16-H), 5.32 (br s, 12-H)]. The triglycosidic structure of **2** was characterized by the HMBC experiment (Fig. 1), in which long-range correlations were observed between the 1‴-proton and the 6″-carbon, between the 1″-proton and the 6′-carbon, and between the 1′-proton and the 23-carbon. Consequently, the structure of gymnemoside-d was elucidated to be 23-*O*-[β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] gymnestrogenin (**2**).

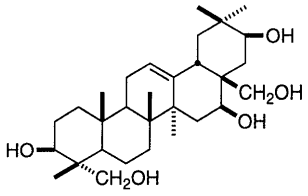
Gymnemoside-e (**3**), obtained as colorless fine crystals of mp 202.8—204.1 °C, liberated D-glucose and D-xylose by acid hydrolysis. The molecular formula $C_{59}H_{98}O_{28}$ of **3** was obtained from quasimolecular ion peaks [m/z 1253 $(M-H)^-$ and 1277 $(M+Na)^+$] in the negative-ion and positive-ion FAB-MS. The ^{1}H-NMR (pyridine-d_5)[7] spectrum of **3** showed signals due to four β-D-glucopyranosyl moieties [δ 4.81 (d, J=7.6 Hz, 1⁗-H), 4.83 (d, J=6.9 Hz, 1′-H), 5.00 (d, J=7.9 Hz, 1″-H), 5.03 (d, J=7.9 Hz, 1⁗′-H)], a β-D-xylopyranosyl moiety [δ 4.92 (d, J=7.3 Hz, 1‴-H)], and the sapogenol moiety [δ 4.04 (m, 23-H_2), 4.16 (m, 3-H), 4.21 (m, 28-H_2), 4.51 (dd-like, 16-H), 5.25 (br s, 12-H)]. The carbon signals of the sapogenol part in the ^{13}C-NMR (Table 1)[7] spectrum of **3** were very similar to those of gymnemasaponins II—V, while the carbon signals of the saccharide moiety resembled those of gymnemasaponin V (**12**), except for the signals due to the β-D-xylopyranoside of **3**. Finally, mild acid treatment of **3** provided gymnemasaponin V (**12**) as the major product. This evidence and the examination of the HMBC experiment (Fig. 1) of **3** led us to formulate the structure of gymnemoside-e as 23-*O*-[β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl]-28-*O*-[β-D-



	R^1	R^2	R^3	R^4
gymnemoside-a (**5**) :	Tig	Ac	H	H
gymnemoside-b (**6**) :	Tig	H	H	Ac
gymnemic acid I (**7**) :	Tig	H	Ac	H
gymnemic acid II (**8**) :	2MB	H	Ac	H
gymnemic acid III (**9**) :	2MB	H	H	H
gymnemic acid IV (**10**) :	Tig	H	H	H
11 :	H	H	H	H



gymnemasaponin V (**12**)



gymmestrogenin (**13**)

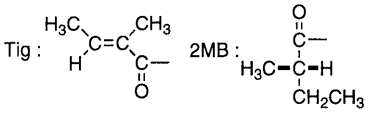


Chart 2

Table 1. ^{13}C-NMR Data of Gymnemosides-c (**1**), -d (**2**), -e (**3**), and -f (**4**)

	1	**2**	**3**	**4**		**1**	**2**	**3**	**4**
C-1	38.8	38.8	38.8	39.0	1‴	131.5			
C-2	26.1	27.4	27.4	27.5	2‴	130.0			
C-3	81.9	72.3	72.4	73.0	3‴	128.9			
C-4	43.5	42.9	42.9	42.9	4‴	133.2			
C-5	47.4	48.7	48.8	48.9	5‴	128.9			
C-6	18.0	18.6	18.6	18.4	6‴	130.0			
C-7	32.5	32.9	32.7	34.7	Ac-1⁗	170.9			
C-8	40.3	40.2	40.2	42.9	2⁗	20.8			
C-9	47.1	47.3	47.2	50.8	Glc-1′		104.8	104.9	104.9
C-10	36.6	37.0	37.0	37.2	2′		75.1	75.1	75.0
C-11	24.0	23.9	23.9	21.1	3′		78.6	78.6	78.5
C-12	124.7	123.0	122.9	26.3	4′		71.7	71.7	71.7
C-13	141.2	143.4	143.7	38.9	5′		77.0	77.0	76.9
C-14	42.5	43.9	44.0	41.0	6′		70.2	70.3	70.0
C-15	36.3	36.7	36.9	38.3	Glc-1″		105.2	105.2	105.0
C-16	67.5	67.8	66.3	75.9	2″		75.0	75.0	74.9
C-17	45.8	43.7	41.4	44.4	3″		78.4	78.4	78.2
C-18	42.7	44.0	44.9	139.5	4″		71.7	71.7	71.5
C-19	45.7	47.8	46.8	133.7	5″		77.1	77.0	76.9
C-20	36.6	36.8	31.0	32.1	6″		70.0	69.9	70.0
C-21	79.8	72.8	34.2	33.4	Xyl-1‴		105.8	105.8	105.7
C-22	71.6	35.0	26.5	28.7	2‴		74.8	74.8	74.7
C-23	64.4	74.8	75.0	74.5	3‴		78.0	78.0	77.9
C-24	13.6	13.2	13.2	12.9	4‴		71.1	71.0	71.0
C-25	16.2	16.2	16.3	17.2	5‴		67.0	67.0	66.9
C-26	17.1	17.1	17.3	16.3	Glc-1⁗			105.7	105.7
C-27	27.5	27.1	27.2	16.0	2⁗			74.8	74.6
C-28	62.3	68.5	77.8	74.6	3⁗			78.6	78.4
C-29	29.3	30.0	33.4	29.9	4⁗			71.5	71.5
C-30	19.8	17.9	24.2	30.4	5⁗			77.2	77.0
GlcA-1′	106.3				6⁗			69.9	69.9
2′	75.5				Glc-1⁗′			105.3	105.1
3′	78.1				2⁗′			75.1	75.1
4′	73.5				3⁗′			78.4	78.2
5′	77.9				4⁗′			71.7	71.6
6′	172.8				5⁗′			78.4	78.1
Bz-1″	166.8				6⁗′			62.8	62.7

The spectra were taken in pyridine-d_5 at 68 MHz.

glucopyranosyl (1→6)-β-D-glucopyranosyl] 23-hydroxylongispinogenin (**3**).

Gymnemoside-f (**4**) was obtained as colorless fine crystals of mp 201.3—203.2 °C, and its IR spectrum was very similar to that of **3**. The negative-ion and positive-ion FAB-MS of **4** showed quasimolecular ion peaks at *m/z* 1253 $(M-H)^-$ and 1277 $(M+Na)^+$, respectively, and the molecular formula $C_{59}H_{98}O_{28}$, which was the same as that of **3**, was determined by high-resolution MS measurement. The ^{1}H-NMR (pyridine-d_5) spectrum of **4** showed signals due to four β-D-glucopyranosyl moieties [δ 4.82 (d, J=7.6 Hz, 1⁗-H), 4.86 (d, J=7.9 Hz, 1′-H),

4.99 (d, $J=7.6$ Hz, 1″-H), 5.00 (d, $J=7.9$ Hz, 1⁗-H)], a β-D-xylopyranosyl moiety [δ 4.92 (d, $J=7.3$ Hz, 1‴-H)], and the sapogenol moiety [δ 0.76, 0.87, 0.96, 0.99, 1.06, 1.08 (all s, 27-, 25-, 24-, 29-, 30-, 26-H_3), 2.55 (dd-like, 13-H), 3.82 (m, 16-H), 3.90 (m, 28-H_2), 4.15 (m, 23-H_2), 4.19 (m, 3-H), 5.25 (s, 19-H)]. The carbon signals of the glycoside moiety in the ^{13}C-NMR (Table 1)[7] of **4** were found to be superimposable on those of **3**, whereas the carbon signals of the sapogenol moiety were similar to those of the olean-18-ene type triterpene glycoside.[9] The structure of the 13β-olean-18-ene sapogenol moiety was confirmed by detailed HMBC and a nuclear Overhauser and exchange spectroscopy (NOESY) experiment. Namely, the HMBC data of **4** showed long-range correlations between the protons and carbons shown in Fig. 1. Furthermore, a NOESY experiment of **4** showed NOE correlations between the following protons: 13-H and 26-H_3, 13-H and 30-H_3, 16-H and 27-H_3, 16-H and 29-H_3, 25-H_3 and 26-H_3. Finally, the glycoside structure of **4** was confirmed by the HMBC experiment, in which long-range correlations were observed between the following protons and carbons: 1‴-H and 6″-C, 1″-H and 6′-C, 1′-H and 23-C, 1⁗-H and 6⁗-C, 1⁗-H and 28-C (Fig. 1). Consequently, the structure of gymnemoside-f was clarified as 23-*O*-[β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl]3β,16β,23,28-tetrahydroxyolean-18-ene (**4**).

Effects of Gymnemosides (1—4), Gymnemic Acids (7—10), and Gymnemasaponin V (12) on Glucose Uptake in Rat Small Intestinal Fragment Recently, we isolated many triterpene glycosides with inhibitory activity on the increase of serum glucose levels in glucose-loaded rats from medicinal foodstuffs[1,2] and natural medicines,[10] and have reported their structure requirement for the activity.[1,2,10] Furthermore, by examination of the inhibitory mechanism, those triterpene glycosides were found to inhibit glucose absorption by suppressing the transfer of glucose from the stomach to the small intestine and by inhibiting the glucose transport system at the small intestinal brush border.[11] We have previously reported the inhibitory activity of the triterpene glycosides from *G. sylvestre* on the increase of serum glucose levels in glucose-loaded rats.[1] In this paper, we examined the effects of the principal triterpene glycosides (**7**—**10**, **12**) and new gymnemosides (**1**—**4**) on the glucose uptake in rat small intestine. As shown in Table 2, gymnemic acids II (**8**) and III (**9**) showed potent inhibitory activities on glucose uptake, which were almost equivalent to those of oleanolic acid 3-*O*-glucuronide[10d] and escin Ia.[10c] Gymnemoside-f (**4**), gymnemic acid IV (**10**), and gymnemasaponin V (**12**) were also found to inhibit the glucose uptake, while gymnemosides-c (**1**), -d (**2**), and -e (**3**) lacked the activity. It is noteworthy that, although *Gymnema* saponin constituents such as gymnemic acids II (**8**) and III (**9**) show no effect on the serum glucose levels in oral glucose-loaded rats,[1] they exhibit potent inhibitory activity on glucose uptake in rat small intestinal fragments. A detailed pharmacological study of *Gymnema* saponin constituents would be an interesting subject for further investigation.

Table 2. Influence of Gymnemosides-c (**1**), -d (**2**), -e (**3**), and -f (**4**) and Known Triterpene Glycosides on Glucose Uptake in Rat Small Intestinal Fragments (*in vitro*)

	Uptake (% of control)		
	0.005 mM	0.05 mM	0.5 mM
Gymnemoside-c (**1**)	110.5± 5.2	115.8± 4.8	102.9± 4.9
Gymnemoside-d (**2**)	117.8± 7.1	119.2± 3.8	100.8± 8.0
Gymnemoside-e (**3**)	107.7± 4.9	100.1±11.7	96.1± 9.2
Gymnemoside-f (**4**)	89.7± 5.5	92.3± 5.9	85.9± 6.5
Gymnemic acid I (**7**)	109.6±15.0	97.3± 4.9	101.2± 8.3
Gymnemic acid II (**8**)	85.6± 6.0	76.9± 7.2	64.7± 2.9**
Gymnemic acid III (**9**)	81.1±15.6	76.5± 9.3	64.2± 9.8
Gymnemic acid IV (**10**)	110.8±13.1	88.3± 0.3	70.4± 0.5*
Gymnemasaponin V (**12**)	88.3± 6.1	92.5± 6.3	89.1± 4.8
Escin Ia	100.3± 5.4	82.9± 4.9	60.2± 2.6**
Oleanolic acid 3-*O*-glucuronide	97.5± 6.4	84.0± 3.6	69.1± 3.9**
		0.1 mM	
Phlorizin		36.8± 4.8**	

*$p<0.05$, **$p<0.01$.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described earlier.[2]

Isolation of Gymnemosides-c (1), -d (2), -e (3), and -f (4) Gymnemosides-c (**1**), -d (**2**), -e (**3**), and -f (**4**) were isolated as described in our previous paper.[1]

Gymnemoside-c (**1**): Colorless fine crystals from aqueous MeOH, mp 211.5—213.0 °C, $[\alpha]_D^{29}$ +6.6° ($c=0.1$, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{45}H_{65}O_{14}$ $(M+H)^+$: 829.4375. Found: 829.4360. Calcd for $C_{45}H_{64}NaO_{14}$ $(M+Na)^+$: 851.4194. Found: 851.4211. UV λ^{MeOH} nm (log ε): 228 (4.3). IR (KBr) cm^{-1}: 3445, 1718, 1649, 1044. ^{1}H-NMR (pyridine-d_5) δ: 0.92, 0.97, 1.01, 1.08, 1.29, 1.33 (3H each, all s, 25-, 24-, 29-, 26-, 30-, 27-H_3), 2.07 (3H, s, OAc), 2.94 (1H, dd-like, 18-H), 3.73, 4.38 (1H each, both d, $J=10.6$ Hz, 23-H_2), 4.28 (1H, m, 3-H), 4.67 (1H, d, $J=10.9$ Hz, 22-H), 4.71, 5.10 (1H each, both d, $J=10.9$ Hz, 28-H_2), 5.19 (1H, dd-like, 16-H), 5.28 (1H, d, $J=7.6$ Hz, GlcA-1′-H), 5.43 (1H, br s, 12-H), 5.96 (1H, d, $J=10.9$ Hz, 21-H), 7.43 (2H, dd, $J=6.9$, 7.3 Hz, Bz-3‴, 5‴-H), 7.53 (1H, d, $J=7.3$ Hz, Bz-4‴-H), 8.30 (2H, d, $J=6.9$ Hz, Bz-2‴, 6‴-H). ^{13}C-NMR (pyridine-d_5) δ_C: see Table 1. Negative-ion FAB-MS *m/z*: 827 $(M-H)^-$. Positive-ion FAB-MS *m/z*: 829 $(M+H)^+$, 851 $(M+Na)^+$.

Gymnemoside-d (**2**): Colorless fine crystals from aqueous MeOH, mp 219.1—221.0 °C, $[\alpha]_D^{29}$ +13.4° ($c=0.1$, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{47}H_{78}NaO_{19}$ $(M+Na)^+$: 969.5035. Found: 969.5050. Calcd for $C_{47}H_{77}Na_2O_{19}$ $(M+2Na-H)^+$: 991.4855. Found: 991.4863. IR (KBr) cm^{-1}: 3410, 1044. ^{1}H-NMR (pyridine-d_5) δ: 0.95, 1.00, 1.03, 1.27 (3H each, all s, 25-, 24-, 26-, 27-H_3), 1.24 (6H, s, 29-, 30-H_3), 2.03, 3.23 (1H each, both dd-like, 22-H_2), 2.56 (1H, dd-like, 18-H), 3.72, 4.48 (1H each, both d, $J=10.6$ Hz, 28-H_2), 3.97 (2H, m, 23-H_2), 4.11 (1H, m, 21-H), 4.18 (1H, m, 3-H), 4.67 (1H, dd-like, 16-H), 4.83 (1H, d, $J=7.6$ Hz, 1′-H), 4.92 (1H, d, $J=7.3$ Hz, 1‴-H), 5.00 (1H, d, $J=7.6$ Hz, 1″-H), 5.32 (1H, br s, 12-H). ^{13}C-NMR (pyridine-d_5) δ_C: see Table 1. Negative-ion FAB-MS *m/z*: 945 $(M-H)^-$. Positive-ion FAB-MS *m/z*: 969 $(M+Na)^+$, 991 $(M+2Na-H)^+$.

Gymnemoside-e (**3**): Colorless fine crystals from aqueous MeOH, mp 202.8—204.1 °C, $[\alpha]_D^{29}$ +14.8° ($c=0.1$, MeOH). High-resolution negative-ion FAB-MS: Calcd for $C_{59}H_{97}O_{28}$ $(M-H)^-$: 1253.6167. Found: 1253.6154. IR (KBr) cm^{-1}: 3410, 1044. ^{1}H-NMR (pyridine-d_5) δ: 0.89, 1.00, 1.02, 1.23 (3H each, all s, 29-, 24-, 26-, 27-H_3), 0.96 (6H, s, 25-, 30-H_3), 1.71 (1H, m), 2.76 (1H, dd-like) (22-H_2), 1.75 (1H, m), 2.19 (1H, dd-like) (15-H_2), 2.33 (1H, dd-like, 18-H), 4.04 (2H, m, 23-H_2), 4.16 (1H, m, 3-H), 4.21 (2H, m, 28-H_2), 4.51 (1H, dd-like, 16-H), 4.81 (1H, d, $J=7.6$ Hz, 1⁗-H), 4.83 (1H, d, $J=6.9$ Hz, 1′-H), 4.92 (1H, d, $J=7.3$ Hz, 1‴-H), 5.00 (1H, d, $J=7.9$ Hz, 1″-H), 5.03 (1H, d, $J=7.9$ Hz, 1⁗-H), 5.25 (1H, br s, 12-H). ^{13}C-NMR (pyridine-d_5) δ_C: see Table 1. Negative-ion FAB-MS *m/z*: 1253 $(M-H)^-$. Positive-ion FAB-MS *m/z*: 1277 $(M+Na)^+$.

Gymnemoside-f (**4**): Colorless fine crystals from aqueous MeOH, mp

201.3—203.2 °C, $[\alpha]_D^{26}$ −8.9° (c=0.1, MeOH). High-resolution negative-ion FAB-MS: Calcd for $C_{59}H_{97}O_{28}$ $(M-H)^-$: 1253.6167. Found: 1253.6193. IR (KBr) cm^{-1}: 3431, 1044. ^{1}H-NMR (pyridine-d_5) δ: 0.76, 0.87, 0.96, 0.99, 1.06, 1.08 (3H each, all s, 27-, 25-, 24-, 29-, 30-, 26-H_3), 1.72 (1H, m), 2.34 (1H, dd-like) (15-H_2), 1.92, 2.45 (1H each, both m, 22-H_2), 2.55 (1H, dd-like, 13-H), 3.82 (1H, m, 16-H), 3.90 (2H, m, 28-H_2), 4.15 (2H, m, 23-H_2), 4.19 (1H, m, 3-H), 4.82 (1H, d, J=7.6 Hz, 1‴-H), 4.86 (1H, d, J=7.9 Hz, 1′-H), 4.92 (1H, d, J=7.3 Hz, 1‴-H), 4.99 (1H, d, J=7.6 Hz, 1″-H), 5.00 (1H, d, J=7.9 Hz, 1⁗-H), 5.25 (1H, br s, 19-H). ^{13}C-NMR (pyridine-d_5) δ_C: see Table 1. Negative-ion FAB-MS m/z: 1253 $(M-H)^-$. Positive-ion FAB-MS m/z: 1277 $(M+Na)^+$.

Acid Hydrolysis of Gymnemosides-c (1), -d (2), -e (3), and -f (4) A solution of gymnemosides (**1**, **2**, **3**, and **4**, 2 mg each) in 5% H_2SO_4–1,4-dioxane (1 : 1, v/v, 1 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was filtered. After removal of the solvent *in vacuo* from their filtrate, the residue was passed through a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (0.02 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N,O*-bis(trimethylsilyl) trifluoroacetamide (0.01 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucuronic acid (i) from **1**, D-glucose (ii) and D-xylose (iii) from **2**, **3**, and **4**. GLC conditions: column, Supelco SPR™-1, 0.25 mm (i.d.) × 30 m; column temperature, 230 °C; t_R, i, 26.7 min; ii, 24.2 min; iii, 19.3 min.

Alkaline Hydrolysis of Gymnemoside-c (1) A solution of gymnemoside c (**1**, 20 mg) in 50% aqueous dioxane (2 ml), was treated with 10% aqueous KOH (2 ml) and the whole was stirred at 37 °C for 1 h. After removal of the solvent from a part (0.1 ml) of the reaction mixture under reduced pressure, the residue was dissolved in $(CH_2)_2Cl_2$ (2 ml) and the solution was treated with *p*-nitrobenzyl-*N,N′*-diisopropylisourea (10 mg), then the whole was stirred at 80 °C for 1 h. The rest of the reaction solution was subjected to HPLC analysis to identify the *p*-nitrobenzyl ester of acetic acid. HPLC conditions: column, YMC-Pack ODS-A (YMC Co., Ltd., Japan), 250 × 4.6 mm (i.d.); solvent, MeOH–H_2O (70 : 30, v/v); flow rate, 1.0 ml/min; t_R, 8.0 min.

The rest of the reaction mixture was neutralized with Dowex HCR W × 2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a product (20 mg) which then was subjected to normal-phase silica-gel column chromatography [BW-200 (Fuji Silysia Chemical Ltd., Japan, 1 g), $CHCl_3$–MeOH (10 : 1)→$CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to give gymnemagenin 3-*O*-glucuronide (**11**, 15 mg) and benzoic acid (3 mg). Compound **11** was identified by comparison of the mp, $[\alpha]_D$, and ^{1}H- and ^{13}C-NMR data with reported values. Benzoic acid was identical to an authentic sample on the basis of TLC and ^{1}H-NMR ($CDCl_3$) comparisons.

Methanolysis of Gymnemoside-d (2) A solution of **2** (10 mg) in 9% HCl–dry MeOH (2 ml) was stirred under reflux for 2 h, then neutralized with IRA-400 (OH^- form) and a filtrate. Work-up of the filtrate yielded a residue which was subjected to normal-phase silica gel column chromatography [1 g, $CHCl_3$–MeOH (20 : 1→10 : 1, v/v)] to furnish gymnestrogenin (**13**, 3 mg). Compound **13** was identified by comparison of the mp, $[\alpha]_D$, and ^{1}H- and ^{13}C-NMR data with reported values.

Partial Methanolysis of Gymnemoside-e (3) A solution of **3** (50 mg) in 9% HCl–dry MeOH (2 ml) was stirred at room temperature (26 °C) for 2 h, then neutralized with IRA-400 (OH^- form) and a filtrate. Work-up of the filtrate yielded a residue which was subjected to HPLC [MeOH–1% aq. AcOH (60 : 40, v/v)] to furnish gymnemoside e (**3**, 18 mg) and gymnemasaponin V (**12**, 13 mg). Compounds **3** and **12** were identical to an authentic sample on the basis of TLC and ^{1}H- and ^{13}C-NMR (pyridine-d_5) comparisons.

Effect of Gymnemosides (1—4), Gymnemic acids (7—10), and Gymnemasaponin (12) on Glucose Uptake in Rat Small Intestinal Fragment Small fragments (0.1—0.15 g) of everted rat intestine were placed in modified Krebs–Henseleit solution, pH 7.4, with ^{14}C-U-glucose (2 mM, 10^5 CPM/ml). Incubation was carried out at 30 °C for 6 min, then the pieces were washed 2 times for 3—5 s with medium containing 1 mM phlorizin without ^{14}C-U-glucose, and placed on filter paper to absorb the water from the tissue. The tissue was then weighed and dissolved using Soluene 350 (Packard Instrument Co., U.S.A.) and the radioactivity was examined.[11] Asterisks denote significant differences from the controls at $^{**}p<0.01$ (n=8).

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