NEW HYPOGLYCEMIC CONSTITUENTS IN "GYMNEMIC ACID" FROM *GYMNEMA SYLVESTRE*

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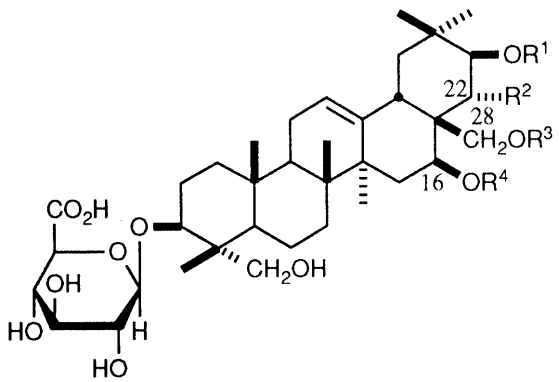
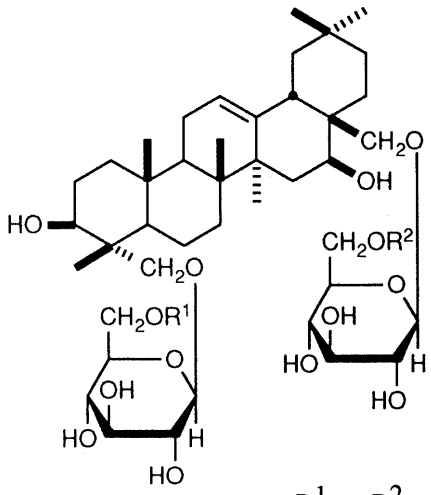
Investigation of hypoglycemic activity of major saponin constituents from "gymnemic acid", a crude saponin fraction of *G. sylvestre*, exposed not only two new saponins, gymnemosides a (**1**) and b (**2**), but also gymnemoside b and gymnemic acid V (**7**) as active principles. Furthermore, an acetyl group linked 16- or 22-hydroxy group in **1** and **2** was found to migrate easily to primary 28-hydroxyl group, while acyl migration from 28-hydroxy group in **3** was little observed.

KEY WORDS gymnemoside; *Gymnema sylvestre*; hypoglycemic activity; Asclepiadaceae; gymnemic acid

The leaves of *Gymnema sylvestre* (Asclepiadaceae), known as "Gur-ma" in Indian folklore, have been used as a stomachic, a diuretic, and a remedy for cough and eye pain. Recently, the crude saponin fraction of this plant named "gymnemic acid" was shown not only to suppress sweet taste sensation but also to inhibit glucose absorption in the small intestine of rats in sucrose tolerance test.[1] As regards antisweet principles in "gymnemic acid", several antisweet saponins were found, and the activities tended to increase with increase in number of acyl groups.[2] However, constituents responsible for inhibition of glucose absorption in "gymnemic acid" have not been clarified. In the previous papers, we have found various saponin constituents inhibiting glucose absorption in the small intestine from natural medicines such as *Aralia elata* SEEM.,[3] *Aesculus hippocastanum* L.,[4] and *Polygala senega* var *latifolia*.[5] We have, therefore, investigated glucose absorption inhibitory saponins in "gymnemic acid" in the course of our studies searching for bioactive saponins in natural medicines. Herein, we describe the saponin constituents inhibiting glucose absorption in "gymnemic acid" and two new saponins, gymnemosides a (**1**) and b (**2**), from the leaves of *G. sylvestre*.



	R^1	R^2	R^3	R^4
Gymnemoside a (**1**)	Tig	OAc	H	H
Gymnemoside b (**2**)	Tig	OH	H	Ac
Gymnemic acid I (**3**)	Tig	OH	Ac	H
Gymnemic acid II (**4**)	MB	OH	Ac	H
Gymnemic acid III (**5**)	MB	OH	H	H
Gymnemic acid IV (**6**)	Tig	OH	H	H
Gymnemic acid V (**7**)	Tig	OTig	H	H
Gymnemic acid VII (**8**)	H	H	H	H
Gymnemagenin 3-*O*-glucuronide (**12**)	H	OH	H	H



	R^1	R^2
Gymnemasaponin II (**9**)	H	H
Gymnemasaponin IV (**10**)	Glc	H
Gymnemasaponin V (**11**)	Glc	Glc

Tig : tigloyl MB : (2*S*)-methylbuthyroyl
Glc : β-D-glucopyranosyl

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The MeOH extract of the leaves of *G. sylvestre* from India was repeatedly separated by reversed-phase and normal-phase SiO_2 column chromatography to give a crude saponin fraction. It was successively purified by reversed-phase SiO_2 column chromatography and HPLC to furnish gymnemosides a (**1**, 0.0083% from leaves) and b (**2**, 0.0046%) along with gymnemic acids I (**3**, 0.012%), II (**4**, 0.0086%), III (**5**, 0.0091%), IV (**6**, 0.0060%), V (**7**, 0.0050%), and VII (**8**, 0.0060%), and gymnemasaponins II (**9**, 0.0025%), IV (**10**, 0.0012%), and V (**11**, 0.016%).

Table 1. ^{13}C NMR Data for Gymnemosides a (**1**) and b (**2**)

	1	**2**		**1**	**2**
C-1	38.7	38.8	C-23	64.4	64.5
C-2	26.1	26.0	C-24	13.6	13.6
C-3	81.9	82.0	C-25	16.2	16.2
C-4	43.5	43.5	C-26	16.9	17.0
C-5	47.4	47.4	C-27	27.4	27.4
C-6	18.0	18.0	C-28	59.9	59.9
C-7	32.5	32.6	C-29	27.4	29.7
C-8	40.2	40.3	C-30	19.7	20.2
C-9	47.1	47.1	C-1'	106.3	106.3
C-10	36.8	36.6	C-2'	75.5	75.5
C-11	23.9	23.9	C-3'	78.1	78.1
C-12	124.3	124.0	C-4'	73.5	73.4
C-13	141.5	141.6	C-5'	78.0	77.9
C-14	42.6	43.0	C-6'	172.8	172.8
C-15	36.6	33.6	C-1"	167.8	167.9
C-16	67.0	69.4	C-2"	128.9	129.7
C-17	47.9	47.4	C-3"	137.9	136.3
C-18	42.6	42.7	C-4"	14.3	14.1
C-19	45.8	46.2	C-5"	12.3	12.1
C-20	36.6	36.7	OAc	170.1	170.3
C-21	76.6	78.8		20.9	21.9
C-22	74.6	70.6			

The spectra were taken in d5-pyridine at 68 MHz.

Gymnemoside a (**1**), colorless fine crystals, mp 207.0~208.5°C, $[\alpha]_D$ +4.7° (MeOH), $C_{43}H_{66}O_{14}$, IR (KBr) : 3453, 1721, 1649, 1040 cm^{-1}, FAB-MS : m/z 829 $(M+Na)^+$ (positive mode), 805 $(M-H)^-$ (negative mode), liberated gymnemagenin 3-*O*-glucuronide (**12**),[2] tiglic acid, and acetic acid upon alkaline treatment (KOH-aq. dioxane). Two acid components were identified as their *p*-nitrobenzyl esters by HPLC analysis. The ^1H NMR (d5-pyridine, 270 MHz, J in Hz)[6] and ^{13}C NMR data (Table 1) assigned by COSY (^1H-^1H, ^1H-^{13}C), HMBC, and HOHAHA (^1H-^1H, ^1H-^{13}C) spectra showed the presence of a sugar residue [δ 5.28 (d, J= 7.6), δ 106.3 (C-1')], a tigloyl group [δ 7.12 (dq-like, 3"-H), δ 1.67 (d, J=6.9, 4"-H_3), δ 1.95 (br s, 5"-H_3), δ 167.8 (C=O)]. Detailed comparison of the ^1H NMR spectrum of **1** with that of **12** disclosed acylation shifts for both the 21-proton [δ 5.74 (d, J=11.2)] and the 22-proton [δ 6.26 (d, J=11.2)] indicating that the two acyl residues were attached at C_{21} and C_{22}. Positional distribution of the two acyl groups was determined by the HMBC spectrum, in which correlations were observed between an acetyl carbonyl carbon and 22-H, and between 1"-C and 21-H. The chemical structure of gymnemoside a (**1**) was thus elucidated as 3-*O*-β-D-glucuronopyranosyl-21-*O*-tigloyl-22-*O*-acetylgymnemagenin.

Gymnemoside b (**2**),[7] colorless fine crystals, mp 211.5~213.0°C, $[\alpha]_D$ +6.6° (MeOH), $C_{43}H_{66}O_{14}$, IR (KBr) : 3445, 1718, 1649, 1044 cm^{-1}, FAB-MS : m/z 829 $(M+Na)^+$ (positive mode), 805 $(M-H)^-$ (negative mode), showed the fairly similar NMR data except for D- and E-ring regions. Alkaline hydrolysis of **2** also gave **12**, acetic acid, and tiglic acid. Inspections on the 2D-NMR spectra (^1H-^1H and ^1H-^{13}C COSY, HMBC) of **2** revealed acylation shifts for the 21-proton [δ 5.74 (d, J=11.0)] and the 16-proton [δ 6.40 (dd-like)] in comparison with **12**. The carbon signals due to C_{21} and C_{16} were shifted downfield, while those due to C_{15} and C_{22} appeared upfield. In the HMBC spectrum, correlations were observed between the following proton and carbon signals : 16-H & $\underline{CO}CH_3$, 21-H & 1"-C. Based on these findings, the structure of gymnemoside b (**2**) was established as shown.

Since the two saponin constituents may be formed from the major saponin in *G. sylvestre*, gymnemic acid I (**3**),[2] during the isolation procedure, acyl migrations of **1**, **2**, and **3** were examined. Both gymnemosides a (**1**) and b (**2**) were heated under reflex in MeOH-1%aq. citric acid (95:5, v/v) or MeOH to give the fraction of **1**, **2**, and **3** in nearly the same ratio, respectively. Prolongation of reaction time increased the proportion of **3**. However, gymnemosides a (**1**) and b (**2**) under HPLC condition [MeOH-1% AcOH (7:3, v/v), r.t.] were unmodified. In contrast, treatment of **3** under reflux in the above media little afforded **1** and **2**. Gymnemosides a (**1**) and b (**2**) would not, therefore, be formed from gymnemic acid I (**3**) but genuine saponins, while **3** may be generated during extraction from **1** and / or **2**.

Table 2 shows inhibitory effects on glucose absorption of gymnemosides a (**1**) and b (**2**), and the major nine saponins (**3**~**11**) from *G. sylvestre* in the glucose tolerance test. With respect to gymnemasaponins II (**9**) and IV (**10**), enough samples to examine the activity were not obtained from the leaves of *G. sylvestre*, so **9** and **10** were prepared from gymnemasaponin V(**11**) by enzymatic hydrolysis using cellulase T-4 (Amano Seiyaku Co., Ltd.) and cellulase from *Aspergillus niger* (Sigma Co., Ltd.), respectively.

Table 2. Inhibitory Effects of Saponin Constituents (100 mg/kg, *p. o.*) from *G. sylvestre* on Elevation of Plasma Glucose Level[9)]

	n	Elevation of Glucose Concentration (mg/dl)		
		0.5 h	1.0 h	2.0 h
control		55.5±4.8	28.9±4.7	11.0±3.4
gymnemoside a (**1**)	5	52.2±2.6	29.3±4.5	10.9±5.6
gymnemoside b (**2**)	4	43.9±7.6*	26.3±6.4	12.1±4.5
gymnemic acid I (**3**)	5	55.5±6.4	26.4±6.2	5.7±2.3
gymnemic acid II (**4**)	5	64.9±3.5	38.4±4.2	16.0±4.2
gymnemic acid III (**5**)	5	40.4±8.0	52.2±4.2	29.3±6.4
gymnemic acid IV (**6**)	5	70.9±5.1	52.1±3.6	17.1±3.7
gymnemic acid V (**7**)	5	35.5±6.1*	19.3±5.0	10.1±2.1
gymnemic acid VII (**8**)	7	42.7±5.2	19.2±6.3	10.6±3.5
gymnemasaponin II (**9**)	5	52.5±4.6	39.2±3.1	23.3±7.5
gymnemasaponin IV (**10**)	3	55.8±1.8	39.8±0.5	19.4±3.7
gymnemasaponin V (**11**)	5	60.0±3.3	40.5±6.9	21.2±4.0

* $p<0.05$

Gymnemoside b (**2**) and gymnemic acid V (**7**) inhibited and gymnemic acid VII (**8**) tended to inhibit elevation of plasma glucose level among the tested saponins.[8)] Although no apparent structure-activity relationship was observed, the three hypoglycemic saponins would be responsible for inhibiting glucose absorption in the small intestine of "gymnemic acid". It is noted that elatosides[3)], escins[4)], and senegasaponins[5)] found in our laboratory are more potent active saponins than gymnemoside b (**2**) and gymnemic acid V (**7**) from "gymnemic acid", which is famous as a glucose absorption inhibitory principle.

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6) The ^{1}H NMR data for **1** : δ 0.90, 0.94, 0.98, 1.03, 1.27, 1.30 (3H each, all s, 25, 26, 24, 29, 30, 27-H_3), 3.73, 4.37 (1H each, both d, J=10.6, 23-H_2), 4.01, 4.26 (2H, ABq, J=11.4, 28-H_2), 5.36 (1H, br s, 12-H).

7) The ^{1}H NMR data for **2** : δ 0.90, 0.93, 0.98, 0.98, 1.21, 1.39 (3H each, all s, 25, 26, 24, 29, 30, 27-H_3), 1.56 (3H, d, J=7.3, 4"-H_3), 1.78 (3H, s, 5"-H_3), 3.73, 4.37 (1H each, both d, J=10.9), 4.07, 4.65 (1H each, both d, J=10.1), 4.88 (1H, d, J=10.9, 22-H), 5.35 (1H, br s, 12-H), 6.92 (1H, dq-like, 3"-H).

8) Gymnemic acid III (**5**) was considered not to inhibit but to delay glucose absorption from the elevation of glucose concentration after 1h and 2h.

9) Each sample was orally administered to male Wistar rats (125-150g) 30 min before oral administration of D-glucose (0.5 g/kg). The control group received oral administration of D-glucose at the same dose.

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