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TWO NEW GYMNEMIC ACID CONGENERS CONTAINING A HEXULOPYRANOSIDE MOIETY

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Two new saponins, gymnemic acid-VIII and -IX, were isolated from the water extract of leaves of Gymnema sylvestre R. Br. along with three known saponins, gymnemic acid III, IV, and V. Their structures, clarified on the basis of spectroscopic and chemical means, present rare examples of a hexulopyranoside moiety at O-3' on gymnemic acid-III and -IV, respectively.

KEYWORDS Gymnema sylvestre; gymnemic acid; gymnemic acid-VIII; gymnemic acid-IX; oxo-glycoside; D-arabino-2-hexulopyranose

The unique property of the leaves of Gymnema sylvestre R. Br. (Asclepiadaceae) that inhibits the ability to taste sweet substances is caused by a mixture of acidic saponins called gymnemic acid (GA),[1)] from which several constituents, gymnemic acids I--VI, were recently isolated and characterized in pure forms.[2)] Their common structural unit is deacylgymnemic acid (DAGA); that is, gymnemagenin 3-O-β-D-glucuronide (**1**).[3,4)] The present paper deals with the isolation of two novel congeners, gymnemic acid-VIII (**6**) and gymnemic acid-IX (**7**); both characteristically contain an oxo-glycoside moiety.

New gymnemic acids, GA-VIII (35 mg) and GA-IX (31 mg), were isolated, together with known saponins, GA-III (**2**), GA-IV (**3**), and GA-V (**4**), from the crude saponin mixture (8 g) obtained by Kurihara's procedure[1b)] from the leaves of G. sylvestre (1.1 kg) by repeated chromatography on an ODS column, and finally purified by preparative HPLC on an ODS column, eluted with a MeOH-0.25% KH_2PO_4 buffer[5)] (Fig. 1). Physical data: GA-VIII (**6**), mp 218-220°C, $[\alpha]_D$ +17.4° (c=0.74, MeOH), $C_{47}H_{74}O_{18}$ [Neg. Fab MS: m/z 925 $(M-1)^-$, 765], IR(KBr): 3450, 1730 cm^{-1}; GA-IX (**7**), mp 222-224°C, $[\alpha]_D$ +11.4° (c=0.7, MeOH), $C_{47}H_{72}O_{18}$ [Neg. Fab MS: m/z 923 $(M-1)^-$, 763], IR(KBr): 3400, 1730, 1700 cm^{-1}.

The ^{13}C-NMR spectra of GA-VIII and GA-IX (Table II) indicated that their aglycones are the same as those of GA-III (**2**) and GA-IV (**3**), respectively. Their sugar portions are the same too, consisting of two hexose derivatives. This was supported by intense peaks at 765 and 763 in the negative Fab MS of GA-VIII and GA-IX, respectively. Those peaks corresponded to the ions split between the first and the

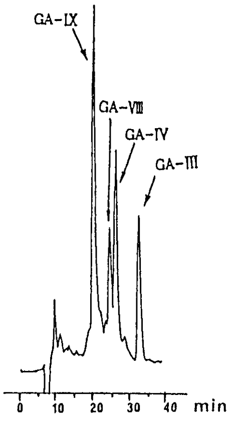


Fig. 1. HPLC Behavior of GA-III, -IV, -VIII, and -IX
Column: TSK gel ODS-120T (4.6× 250mm).
Detector: RI. Flow rate: 0.5 ml/min.
Solvent: MeOH/0.25%KH_2PO_4 (62/38).

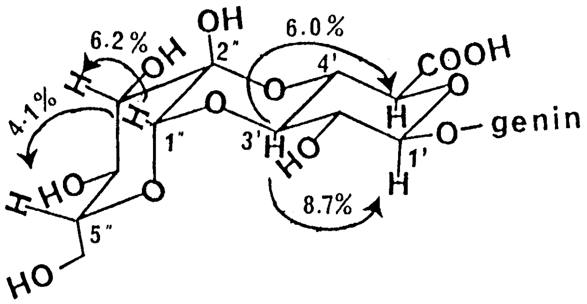
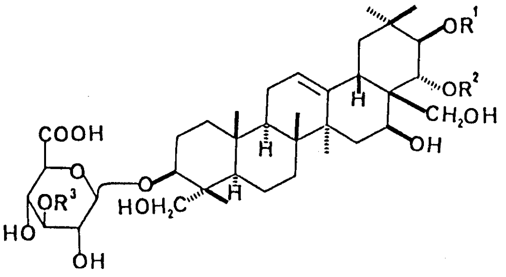


Fig. 3. Structures of **6a** and **7a**

Table I. Known and New Gymnemic Acids

Comp.	Name	R^1	R^2	R^3
1	DAGA	H	H	H
2	GA-III	MB	H	H
3	GA-IV	Tig	H	H
4	GA-V	Tig	Tig	H
5	GA-VI	Tig	H	β-Glc
6	GA-VIII	MB	H	OG[a]
7	GA-IX	Tig	H	OG[a]
8		Tig	H	β-Man

a) The oxo-form is indicated.

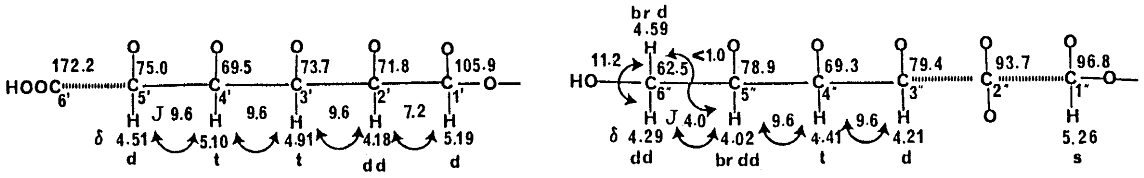


MB= [S]-2-Methylbutyloyl
Tig= Tigloyl
OG= β-D-<u>arabino</u>-2-Hexulopyranosyl

Table II. ^{13}C-NMR Chemical Shifts of Gymnemic Acids and Derivatives[a)]

Comp.	2	3	4	6	7	8
Aglycone						
2	26.0	26.0	26.0	26.0	25.9	26.0
3	81.8	81.0	81.8	82.1	81.8	81.7
4	42.6	42.6	42.7	42.6	42.5	42.6
15	36.3	36.2	36.8	36.2	35.9	36.2
16	68.0	68.0	67.0	68.0	68.0	68.0
17	47.0	47.1	48.0	47.0	47.0	47.1
18	42.0	42.0	42.7	42.0	41.9	42.0
19	46.2	46.2	45.9	46.2	46.2	46.2
20	36.4	36.6	36.7	36.4	36.5	36.6
21	79.1	79.6	76.6	79.0	79.6	79.6
22	71.2	71.2	74.6	71.2	71.1	71.2
23	64.4	64.4	64.4	64.1	64.0	64.1
24	13.6	13.6	13.6	13.6	13.5	13.6
28	58.1	58.1	59.9	58.1	58.0	58.1
Glucuronic acid moiety						
1'	106.1	105.5	106.1	106.3	105.9	105.6
2'	75.4	75.1	75.4	72.1	71.8	74.4(-0.7)[b)]
3'	78.1	78.2	78.1	73.8	73.7	85.1(+6.9)
4'	73.4	73.5	73.4	69.6	69.5	71.9(-1.6)
5'	77.8	77.4	77.6	75.2	75.0	76.8
6'	172.9	173.8	172.9	171.5	172.2	172.8
Second sugar moiety						
1"				97.0	96.8	102.3
2"				93.8	93.7	71.6
3"				79.7	79.4	75.4
4"				69.5	69.3	68.4
5"				79.4	78.9	78.8
6"				62.8	62.5	62.3
Acyl Group						
1	176.6	168.2	167.6, 167.8	176.6	168.5	168.3
2	42.0	129.7	128.9, 128.9	42.0	129.6	129.7
3	27.2	136.4	137.6, 138.0	27.2	136.8	136.6
4	12.0	12.4	12.2, 12.2	12.0	12.4	12.4
5	17.1	14.1	14.1, 14.2	17.1	14.2	14.2

a) Chemical shift (δ) in pyridine-d_5 with a few drops of D_2O with TMS as an internal standard.
b) Parenthetical values indicate Δδ = δ (Comp. 8)-δ (Comp. 3).



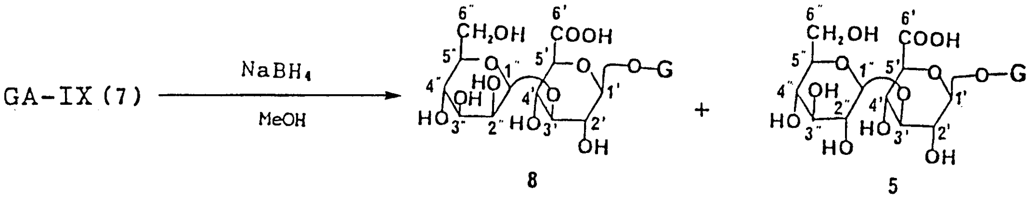
1st sugar portion 2nd sugar portion

—— connectivity detected , ……connectivity not clarified

Fig. 2. Connectivity of Sugar Portions in GA-IX (7)

second sugar moiety. Connectivity in the sugar portion was clarified by finding the correlation peaks in the ^{13}C-^{1}H and ^{1}H-^{1}H COSY spectra of GA-IX, which lead to the sequence shown in Fig. 2. It is notable that in the second sugar moiety, the anomeric proton appears as a singlet (δ5.26) and the sugar has a singlet carbon signal at δ93.7, the chemical shift of which corresponds to a hydrate (or acetal) form of an oxo-glycoside.[6] We therefore consider that GA-IX has a glucuronide connected with an *arabino*-2-hexulose.

Reduction of GA-IX with sodium borohydride in methanol afforded a mixture. The ^{13}C-NMR peaks of the major product were assigned as those of GA-IV 3'-*O*-β-D-mannopyranoside (**8**), since the chemical shifts of the second sugar moiety were almost identical with those of methyl β-D-mannopyranoside,[7] and the glycosylation shifts on the glucuronide portion of this compound indicated that mannose was connected with *O*-3' as a β-glycoside. The minor product was assigned as GA-IV-3'-*O*-β-D-glucopyranoside (**5**), since its carbon chemical shifts were identical with those of GA-VI (**5**) reported by Yoshikawa *et al.*[2b] The above assignment was confirmed by methanolysis (6% HCl-MeOH, 80°C) of the reduction product leading to a mixture of methyl (α and β) D-glucoside, methyl (α and β) D-mannoside, and methyl glucuronide methyl ester (three peaks) in a ratio of *ca.* 1:3:4 (GLC of the trimethylsilyl derivatives).



Gymnemic acid-VIII and -IX are therefore concluded as 3'-*O*-β-D-*arabino*-2"-hexulopyranosyl-GA-III (**6**) and 3'-*O*-β-D-*arabino*-2"-hexulopyranosyl-GA-IV (**7**), respectively.

The carbon signal at δ93.8 (or 93.7) and higher field shifts of the glucuronide carbon signals in GA-VIII (or GA-IX), when compared with those of **8**, suggest that the isolated compound is an intramolecular hemiacetal.[8] Among four possible hemiacetal structures expected from those in which the carbonyl connects with *O*-2' or *O*-4' of glucuronide and with the equatorial or axial stereochemistry for the newly formed ether linkage, we suggest that the isolated compound should be of the most stable form, **6a**, since the hemiacetals of oxo-glycosides are known to equilibrate with the oxo-form.[9] In support of the structure **6a**, NOE experiments of GA-VIII (see Fig. 3) showed that both glucuronide and oxoglycosyl rings are of *C1* conformation, and there were no NOE between H-1" and H-3' and between H-1" and H-4'.[10] GA-IX also had the structure **7a**.

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5) They are hardly separable from GA-III and GA-IV, respectively, by the solvent MeOH-H_2O or MeOH-0.8%$(NH_4)_2CO_3$ buffer, but can be separable by this solvent system.
6) Methyl β-D-*arabino*-2-hexulopyranoside and methyl β-D-*ribo*-3-hexulopyranoside, in D_2O, showed the hydrated carbonyl signals at δ94.0 and 94.7, respectively (unpublished observations in our laboratory).
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10) These results do not necessarily confirm the structure **6a**. The genuine forms of GA-VIII and GA-IX in the plant must also be a future problem.

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