Tetrahedron Letters,Vol.30,No.9,pp 1103-1106,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

STRUCTURE STUDIES OF NEW ANTISWEET CONSTITUENTS FROM GYMNEMA SYLVESTRE [1]

Kazuko Yoshikawa*[a], Kayoko Amimoto[a], Shigenobu Arihara[a], and Kouji Matsuura[b]

[a]Faculty of Pharmaceutical Sciences, Tokushima-Bunri University, Tokushima-shi, Tokushima, 770, Japan

[b]Teikoku Seiyaku Co., Ouchi-cho, Ookawa-gun, Kagawa, 769-26, Japan

Summary : Gymnemic acid I , II , III and IV have been isolated from hot water extract of leaves of Gymnema sylvestre R. Br. as antisweet principles. The whole structures were determined by chemical and spectroscopic means.

Gur-ma (an Indian word meaning sugar destroying), being the leaves of Gymnema sylvestre R. Br. (Asclepiadaceae), one of the traditional medicines used in India as stomachic, diuretic, cough remedy, etc. are well known to have a specific feature of temporarily[2] destroying the taste of sweetness. Subsequently, the antiviral effect[3] and strong reducing effect on blood sugar concentration[2,4] were found in gymnemic acid (mixture)[5] which was isolated as an antisweet principle. However, its chemical structure has not yet become clear[3,6] Therefore, we started to isolate gymnemic acid and elucidate the structure.

Hot water extract of the dry leaves (1.5 kg) of G. sylvestre R. Br. followed by treatment with Amberlite XAD-2 column chromatography gave a saponin fraction. Repeated separation of saponin fraction by reversed-phase and ordinary-phase SiO_2 column chromatography furnished four new antisweet principles, gymnemic acid I (1, 100 mg), II (2, 240 mg), III (3, 40 mg), IV (4, 70 mg).

Mild acid hydrolysis of 1-4 furnished glucuronic acid (glcUA) and gymnemagenin (6)[7], mp 313-314°C, $[\alpha]_D$ + 53.5°(c 1.8, MeOH), $C_{30}H_{50}O_6$ [FABMS m/z 530(M+Na+H)$^+$]. Alkaline hydrolysis of 1-4 furnished prosapogenin (5), mp 230-231°C, $[\alpha]_D$ + 8.4° (c 1.8, MeOH), $C_{36}H_{58}O_{12}$ [FABMS m/z 728(M+2Na)$^+$], which, on acid hydrolysis, provided 6 and glcUA.

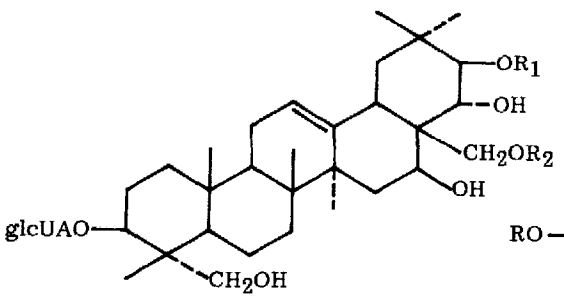
By comparing the ^{13}C-NMR spectrum of 5 with that of 6 a glycosylation shift[8] of + 8.2 ppm was observed at C_3, disclosing the site of glycosylation in 5. Hence 5 is 3-O-β-D-

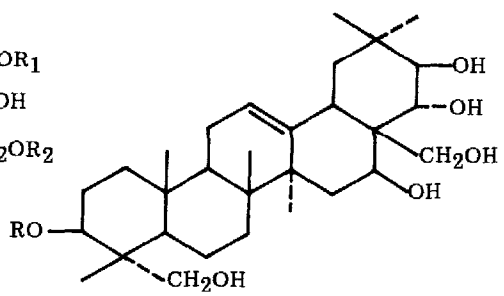
glucuronopyranosyl gymnemagenin[9].

Gymnemic acid II (2), mp 212-213°C, $[\alpha]_D$ + 36.3° (c 1.5, MeOH), possessed the molecular formula $C_{43}H_{68}O_{14}$. ^{1}H-NMR spectrum of 2 suggested that 2 was composed of one mol each of 6, acetic acid, 2-methylbutyric acid[10] and glcUA. Acetic acid and 2-methylbutyric acid obtained by mild alkaline hydrolysis of 2 were identified as their p-nitrobenzyl esters. [HPLC, YMAC-Pack C_8, 6ϕ, 15 cm, 60% MeOH). The absolute configuration of 2-methylbutyric acid was determined as S by optical rotation ($[\alpha]_D$ + 16.3°(c 0.3, 50% MeOH), Lit.$[\alpha]_D$ +19.2°(c 1.0, EtOH).[11]

By comparison of 2 with that of 5 in both ^{1}H- and ^{13}C-NMR spectra, two acylation shifts were observed at the C_{21} (position)[+ 0.79 ppm(21-H), + 1.0 ppm(C-21)] and the C_{28}(position) [+ 0.28 and 0.52 ppm(28-H_2), +3.7 ppm(C-28)][12]. Therefore, in 2, the O-21 and O-28 of 5 should be acylated. Long rang selective proton decoupling (LSPD)[13] experiment revealed that 21-H (δ 5.68) had coupled to C-1''(δ 176.6) and 28-H_2(δ 4.61 and 5.02) had coupled to C-1''' (δ 170.9), establishing the existence of 2-methylbutyloyl at the C_{21}(position) and acetyl at the C_{28}(position). THe structure of 2 is thus 3-O-β-D-glucuronopyranosyl-21-[S(+)-2-methylbutyloyl]-28-O-acetyl gymnemagenin.

^{1}H-NMR spectrum of 1, $C_{43}H_{66}O_{14}$, mp 211-212°, $[\alpha]_D$ + 36.7° (c 2.4, MeOH),suggested that 1 was composed of one mol each 6, acetic acid, tiglic acid and glcUA. Acetic acid and tiglic acid were converted to p-nitrobenzyl esters as in 2 and identified using HPLC. Comparison of the ^{1}H- and ^{13}C-NMR spectrum of 1 with those of 5 disclosed C_{21}[+ 0.89 ppm (21-H), + 1.7 ppm(C-21)] and the C_{28}[+ 0.34 and + 0.56 ppm(28-H_2), + 4.0 ppm(C-28)] as acylation sites in the former. The LSPD experiment showed the presence of tigloyl at the C_{21} (position) and acetyl at the C_{28}(position). The structure of 1 is thus 3-O-β-D-glucurono-





	R_1	R_2
1	tigloyl	acetyl
2	2-methylbutyloyl	acetyl
3	2-methylbutyloyl	H
4	tigloyl	H

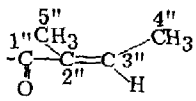
	R
5	glcUA
6	H

Table I. ^{1}H-NMR data for 1-6 (C_5D_5N/D_2O, Me_4Si=0)

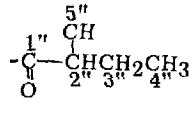
	H-16	H-21	H-22	H_2-23	H_2-28	Acyl moieties	Anomeric H
1	5.14dd J=11.5,5.0	5.78d J=10.5	4.59d J=10.5	3.71,4.37d J=10.5	4.65,5.08d J=11.0	1.64d,1.91s,7.07q J=6.5 J=6.5	5.29d J=7.5
2	5.10dd J=10.0,5.0	5.68d J=10.5	4.54d J=10.5	3.70,4.36d J=11.0	4.61,5.02d J=11.0	0.98t,1.26d,1.54q J=7.0 J=7.0 J=7.0 2.59sex,1.97s J=7.0	5.27d J=8.0
3	5.09dd J=10.5,5.0	5.70d J=10.5	4.94d J=10.5	3.71,4.35d J=11.0	4.06,4.67d J=10.5	0.99t,1.27d,1.54q J=7.0 J=7.0 J=7.0 2.58q J=7.0	5.27d J=8.0
4	5.12dd J=11.5,5.0	5.79d J=10.5	5.03d J=10.5	3.71,4.35d J=10.0	4.08,4.70d J=10.0	1.64d,1.90s,7.08q J=7.5 J=7.5	5.27d J=8.0
5	5.07dd J=11.0,5.0	4.89d J=10.5	4.07d J=10.5	3.71,4.37d J=11.0	4.09,4.74d J=10.5		5.28d J=8.0
6	5.08dd J=11.5,5.0	4.90d J=10.5	4.08d J=10.5	3.72,4.18d J=10.5	4.12,4.75d J=10.5		

Table II. ^{13}C-NMR data for 1-6 (C_5D_5N, Me_4Si=0)

		1	2	3	4	5	6
Aglycone moiety	C-2	26.3	26.1	26.1	26.1	26.1	27.7
	C-3	82.3	81.9	81.9	81.9	82.0	73.8
	C-4	43.7	43.5	43.5	43.5	43.6	42.9
	C-15	36.4	36.4	36.4	36.3	35.8	36.0
	C-16	67.7	67.5	68.0	68.1	68.4	67.8
	C-17	45.9	45.6	47.1	47.2	46.6	46.6
	C-20	36.9	36.4	36.5	36.8	36.7	36.8
	C-21	79.1	78.4	79.1	79.7	77.4	77.3
	C-22	71.7	71.4	71.2	71.3	73.5	73.3
	C-23	64.4	64.3	64.4	64.4	64.4	68.3
	C-24	13.9	13.6	13.6	13.7	13.7	13.1
	C-28	62.6	62.3	58.1	58.2	58.6	58.6
3-O-β-D-Glucurono-pyranosyl moiety	C-1'	106.3	106.3	106.3	106.3	106.2	
	C-2'	75.5	75.5	75.5	75.5	75.5	
	C-3'	78.1	78.1	78.1	78.1	78.2	
	C-4'	73.5	73.4	73.5	73.5	73.8	
	C-5'	77.8	78.0	77.9	77.9	77.9	
	C-6'	173.1	172.9	172.9	172.9	173.0	
Tigloyl or 2-methyl-butyloyl moiety	C-1''	168.5	176.6	176.6	168.3		
	C-2''	129.7	42.1	42.1	129.7		
	C-3''	137.3	27.3	27.3	136.6		
	C-4''	12.7	12.1	12.1	12.5		
	C-5''	14.6	17.2	17.2	14.3		
Acetyl moiety	C-1'''	171.4	170.9				
	C-2'''	21.1	20.7				



tigloyl



2-methylbutyloyl

pyranosyl-21-O-tigloyl-28-O-acetyl gymnemagenin.

Using a similar strategy, the structures of 3, mp 218-219°C, $[\alpha]_D$ + 7.6° (c 2.9, MeOH) and of 4, mp 220-221°C, $[\alpha]_D$ + 8.8° (c 5.4, MeOH) have been assigned. A 0.5 mM each of gymnemic acid I, II and 1 mM each of gymnemic acid III, IV solution led to a complete suppression of sweetness induced by 0.4 mM sucrose. In general, we have found the antisweet activity of these saponins decreases with the decreasing number of acyl groups. 5 and 6 were not active at all.

- Reference and Note -

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(Received in Japan 16 December 1988)