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LICORICE-SAPONINS A3, B2, C2, D3, AND E2, FIVE NEW OLEANENE-TYPE TRITERPENE OLIGOGLYCOSIDES FROM CHINESE GLYCYRRHIZAE RADIX

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Ten new oleanene-type triterpene oligoglycosides were isolated from Chinese Glycyrrhizae Radix, the dried root of Glycyrrhiza uralensis Fischer [Tohoku-Kanzo (in Japanese) from China], and the structures of five oligoglycosides, named licorice-saponins A3 (3), B2 (5), C2 (7), D3 (9), and E2 (12), have been determined on the basis of chemical and physicochemical evidence.

KEYWORDS —— Glycyrrhizae Radix; Glycyrrhiza uralensis; licorice-saponin A3; licorice-saponin B2; licorice-saponin C2; licorice-saponin D3; licorice-saponin E2; oleanene-type triterpene oligoglycoside

Glycyrrhizae Radix (licorice root, the root of Glycyrrhiza sp.) is a Chinese crude drug most abundantly used in Japan for many purposes and it has been extensively investigated to shed light on its bioactive principles.[1] Among triterpene oligoglycosides, which are the principal ingredients of Glycyrrhizae Radix, glycyrrhizin (2) is the most important principle. Currently, 2 and its derivatives are used clinically to treat gastric ulcer, allergic symptoms, and liver disease. Many other biological activities of 2 and its derivatives, such as mineral corticoid-like action (pseudo-aldosteronism), inhibition of virus growth, inactivation of virus particles, interferon-inducing activity, and antitumor-promoting activity, have been reported.[1,2] But no work has been reported on the chemical characterization of the triterpene oligoglycosides other than glycyrrhizin (2). Only the sapogenols, which were obtained by acidic hydrolysis of the glycosidic mixture, have been investigated.[1a,b]

As a part of our chemical characterization studies of crude drug processing,[3] we have compared the chemical constituents of Glycyrrhizae Radix of various origins. This paper describes the structure of licorice-saponins A3 (3), B2 (5), C2 (7), D3 (9), and E2 (12), which were isolated together with licorice-saponins F3, G2, H2, I2, J2[4] from Chinese Glycyrrhizae Radix [Tohoku-Kanzo (in Japanese) from China],[5] the dried root of Glycyrrhiza uralensis Fischer (Leguminosae).[6]

The MeOH extract of Radix was partitioned into an AcOEt-H_2O mixture and the H_2O-soluble portion was first subjected to reversed-phase silica gel column chromatography (Bondapak C_{18}, H_2O-MeOH) to separate the oligoglycoside fraction. Repeated separation of the oligoglycoside fraction by ordinary-phase silica gel column chromatography ($CHCl_3$-MeOH-H_2O) and subsequent HPLC (Zorbax BP-ODS, CH_3CN-1% aq.AcOH), furnished, together with 2 and known flavonoid glycosides, licorice-saponins A3 (3), B2 (5), C2 (7), D3 (9), E2 (12), F3, G2, H2, I2, J2 (0.029, 0.004,

0.005, 0.007, 0.012, 0.002, 0.022, 0.003, 0.002, and 0.002 %, respectively from the crude drug) (2 in 3.608 %).

Licorice-saponin A3 (3), mp 196-199°C, $[\alpha]_D^{23}$ +69° (MeOH), $C_{48}H_{72}O_{21}\cdot 3H_2O$,[7] λ_{max}^{MeOH} nm(ε): 249 (8800), has carboxyl groups (1716 cm^{-1}) and ester and enone groups (1741, 1650 cm^{-1}) as shown by its IR spectrum (KBr). Methanolysis of 3 with 9% HCl-dry MeOH yielded methyl glucoside, methyl glucuronide and glycyrrhetic acid (1) while alkaline hydrolysis of 3 furnished glycyrrhizin (2). Methylation of 3 with ethereal CH_2N_2 in MeOH provided a dimethyl ester (3a), mp 205-208°C, $[\alpha]_D^{23}$ +75° (MeOH), $C_{50}H_{76}O_{21}\cdot H_2O$, λ_{max}^{MeOH} nm(ε): 249 (8900), IR (KBr): 3420, 1740, 1650 cm^{-1}. Treatment of 3a with $NaBH_4$ in MeOH furnished 3b, mp 223-224°C, $[\alpha]_D^{19}$ +20° (MeOH), $C_{48}H_{76}O_{19}\cdot 4H_2O$, λ_{max}^{MeOH} nm(ε): 249 (10700), IR (KBr): 3420, 1741, 1652 cm^{-1}, which, on methanolysis, afforded methyl glucoside and 1. Based on these findings and the ^{13}C NMR examinations of 3a and 3b, licorice-saponin A3 (3) was assumed to be a 30-β-D-glucopyranosyl derivative of glycyrrhizin (2). That assumption has been finally verified by the following synthesis from 2.

Methylation of 2 with 1% HCl-dry MeOH provided a 6',6''-dimethyl ester (2a, 95 %), a white powder, $[\alpha]_D^{20}$ +45° ($CHCl_3$), $C_{44}H_{66}O_{16}$, λ_{max}^{MeOH} nm(ε): 249 (10200), IR ($CHCl_3$): 3300, 1750, 1710, 1651 cm^{-1}, ^1H NMR (500 MHz, d_5-pyridine-D_2O, δ): 3.69, 3.81 (3H each, both s, COOMe x2), 4.96, 5.38 (1H each, both d, J=8 Hz, anomeric H x2). Acetylation of 2a with Ac_2O in pyridine gave 2b, a white powder, $[\alpha]_D^{20}$ +45° ($CHCl_3$), λ_{max}^{MeOH} nm(ε): 249 (10200), IR ($CHCl_3$): 1750, 1710, 1651 cm^{-1}. Glycosidation of 2b with 1-bromo-2,3,4,6-tetra-O-acetylglucose and $Hg(CN)_2$ in benzene furnished 3c (82 %), a white powder, $[\alpha]_D^{20}$ +43° ($CHCl_3$), $C_{68}H_{94}O_{30}$, λ_{max}^{CHCl3} nm(ε): 249 (6500), ^1H NMR (500 MHz, d_5-pyridine, δ): 3.70, 3.80 (3H each, both s, COOMe x2), 4.95, 5.33, 6.33 (1H each, all d, J=8 Hz, anomeric H x3). Deacetylation of 3c with 0.1% NaOMe-MeOH (20°C, 0.5 h), followed by hydrolysis with 0.3% K_2CO_3-50% aq.EtOH (20°C, 2 h), gave 3 (62 %)

Diazomethane methylation of licorice-saponin B2 (5), mp 209-210°C, $[\alpha]_D^{19}$ +54° (MeOH), $C_{42}H_{64}O_{15}\cdot H_2O$, IR (KBr): 3400, 2950, 1720 cm^{-1}, furnished a trimethyl ester (5a), mp 169-172°C, $[\alpha]_D^{20}$ +51° (MeOH), $C_{45}H_{70}O_{15}\cdot 2H_2O$, IR (KBr): 3350, 2915, 1720 cm^{-1}. The ^{13}C NMR spectrum of 5a closely resembled the spectrum of 2a[8] except for some signals due to the deoxo-sapogenol moiety. Methanolysis of 5a liberated methyl glucuronide and 4a.[9] The structure of 5 was finally confirmed by identification with deoxoglycyrrhizin[10] which was prepared from glycyrrhizin (2).

Licorice-saponin C2 (7), mp 249-251°C, $[\alpha]_D^{21}$ -120° (MeOH), $C_{42}H_{62}O_{15}\cdot 3H_2O$, IR (KBr): 3400, 1710, 1640 cm^{-1}, showed a UV maximum (MeOH, ε) at 241 nm (14100), 249 (15800), 259 (10200) ascribable to a conjugated heteroannular diene moiety. Diazomethane methylation of 7 yielded a trimethyl ester (7a), mp 174-176°C, $[\alpha]_D^{23}$ -110° (MeOH), $C_{45}H_{68}O_{15}\cdot 2H_2O$, λ_{max}^{MeOH} nm(ε): 242 (13000), 250 (14600), 259 (9300), which, on methanolysis, gave methyl glucuronide and 6a.[11] Treatment of 7a with $NaBH_4$ in MeOH and subsequent permethylation of the reaction product with MeI/DMSO/NaH followed by methanolysis, liberated methyl 2,3,4,6-tetra-O-methylglucopyranoside and methyl 3,4,6-tri-O-methylglucopyranoside. Finally, the ^{13}C NMR examination of 7a has led to the formulation of licorice-saponin C2 (7).

Methanolysis of licorice-saponin D3 (9), a white powder, $[\alpha]_D^{20}$ -5° (MeOH), $C_{50}H_{76}O_{21}$, IR (KBr): 3400, 1730, 1712 cm^{-1}, furnished methyl glucuronide, methyl rhamnoside, and a new sapogenol (8), a white powder, $[\alpha]_D^{23}$ +72° ($CHCl_3$), $C_{32}H_{50}O_5$.

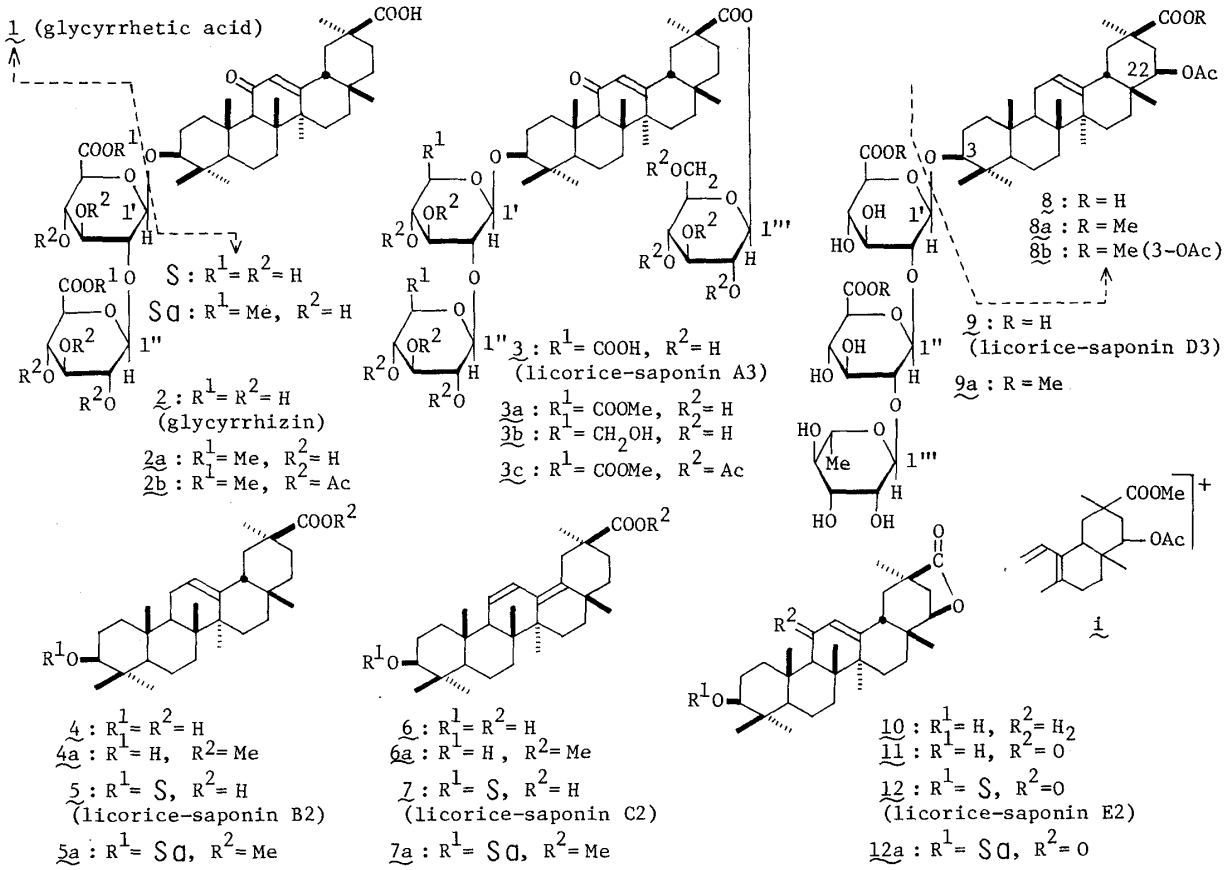

Table. ^{13}C NMR Data for 3a, 3b, 5a, 7a, 9a, and 12a (at 22.5 MHz, in d_5-Pyridine, δ_C)

		3a	3b	5a	7a	9a	12a
Sapogenol moiety	C-3	89.0	88.6	89.5	89.5	89.9	89.0
	C-11	199.3	199.5	47.8	125.5[a]	47.6	198.8
	C-12	128.2	128.4	122.9	126.7[a]	122.2	129.6
	C-13	169.1	169.1	144.7	135.8	143.6	164.3
	C-18	47.9	47.9	48.6	135.8	47.6	44.8
	C-22	39.4	39.6	38.7	39.9	77.5[c]	84.0[a]
	C-30	175.4	175.5	177.3	178.4	177.2	179.3
3-O-β-D-Glucurono- or gluco-pyranosyl moiety	C-1'	104.3	104.6	105.0	104.8	104.7	104.6
	C-2'	83.7	82.8	84.4	84.3	79.1	83.8[a]
	C-3'	76.0[a]	77.5[a]	76.3a]	76.5	76.4[a]	76.2[b]
	C-4'	72.2[b]	71.3	72.6[b]	72.4[b]	72.2[b]	72.2[c]
	C-5'	77.0	77.5[a]	77.4[c]	77.2[c]	77.9[c]	77.2
	C-6'	169.6[c]	62.4[c]	170.1[d]	169.8[d]	169.6[d]	169.7[d]
2'-O-β-D-Glucurono- or gluco-pyranosyl moiety	C-1"	106.1	105.4	106.8	106.6	102.4	106.3
	C-2"	75.8[a]	76.5	76.4[a]	76.5	78.2	76.1[b]
	C-3"	77.0	77.7[a]	77.6[c]	77.4[c]	76.7[a]	77.2
	C-4"	72.3[b]	71.3	72.9[b]	72.7[b]	72.9[b]	72.5[c]
	C-5"	77.0	77.5[a]	76.7[a]	77.2[c]	77.5[c]	77.2
	C-6"	169.7[c]	62.4[c]	170.3[d]	170.1[d]	169.8d]	169.8[d]
30-O-β-D-Gluco- or 2"-O-α-L-rhamno-pyranosyl moiety	C-1"'	95.4	95.5			101.6	
	C-2"'	73.6	73.7			71.9	
	C-3"'	78.7	78.9			72.9	
	C-4"'	70.7	70.9			73.9	
	C-5"'	78.1	78.2			69.2	
	C-6"'	61.8	62.0[c]			18.5	

a), b), c), d), e), f) Assignments may be interchangeable within the same column.

Diazomethane methylation of 8 gave a methyl ester (8a), mp 232-234°C, $[\alpha]_D^{23}$ +68° ($CHCl_3$), $C_{33}H_{52}O_5$, IR ($CHCl_3$): 3611, 2941, 1748, 1721 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$, δ): 1.98 (3H, s, OAc), 3.68 (3H, s, COOMe), 3.24 (1H, dd, J=6,7 Hz, 3α-H), 4.61 (1H, dd, J=3,3 Hz, 22α-H), 5.40 (1H, t-like, 12-H), MS (%): m/z 528 (M^+, 5), 468 (M^+-AcOH, 100), 320 (i, 8), 260 (i-AcOH, 45). Acetylation of 8a with Ac_2O-pyridine, gave a diacetate (8b), mp 201-203°C, $[\alpha]_D^{22}$ +68° ($CHCl_3$), $C_{35}H_{54}O_6$, ^1H NMR (500 MHz, $CDCl_3$, δ): 4.51 (1H, dd, J=7,9 Hz, 3α-H), 4.60 (1H, dd, J=3,3 Hz, 22α-H), MS (%): m/z 570 (M^+, 4), 320 (i, 8), 260 (i-AcOH, 40). Acidic treatment of 8a with 10% H_2SO_4-50% aq.MeOH gave deoxoglabrolide (10).[12] Based on these findings and the ^1H NMR data comparison of 8a, 8b and 10, the structure of new sapogenol has been determined as 8 having a 22β-acetoxyl moiety.

Permethylation of 9 followed by $NaBH_4$ treatment and methanolysis, gave methyl 3,4-di-O-methylglucopyranoside and methyl 2,3,4-tri-O-methylrhamnopyranoside in 2:1 ratio. Finally, the ^{13}C NMR data for 9a (prepared by CH_2N_2 treatment of 9) including the ^{13}C-^1H coupling constants of anomeric C signals [171 Hz (rhamnoside), 160 Hz (glucuronide x2)], has corroborated the structure of licorice-saponin D3 (9) as shown.

Diazomethane methylation of licorice-saponin E2 (12), mp 216-219°C, $[\alpha]_D^{23}$ +68° (MeOH), $C_{44}H_{64}O_{16}\cdot 2H_2O$, λ_{max}^{MeOH} nm(ε): 250 (12700), furnished a dimethyl ester (12a), mp 232-234°C, $[\alpha]_D^{21}$ +65° (MeOH), $C_{46}H_{68}O_{16}\cdot 3H_2O$, λ_{max}^{MeOH} nm(ε): 250 (11000). Methanolysis of 12a liberated methyl glucuronide and glabrolide (11).[12] The result from the $NaBH_4$ treatment of 12a, and subsequent methylation analysis and ^{13}C NMR examination of 12a, have finally substantiated the structure of licorice-saponin E2 (12).

We have also compared the oligoglycosidic constituents of various Glycyrrhizae Radix and found that the above-mentioned licorice-saponins are commonly distributed in Chinese Glycyrrhizae Radix. The biological activities of these licorice-saponins are under investigation.

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