LICORICE-SAPONINS F3, G2, H2, J2, AND K2, FIVE NEW OLEANENE-TRITERPENE OLIGOGLYCOSIDES FROM THE ROOT OF GLYCYRRHIZA URALENSIS

Isao KITAGAWA,* Jun Liang ZHOU, Masahiro SAKAGAMI, Emiko UCHIDA, and Masayuki YOSHIKAWA

Faculty of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565, Japan

Following the structure elucidation of licorice-saponins A3, B2, C2, D3, and E2, the structures of five more new oleanene-triterpene oligoglycosides named licorice-saponins F3 (1), G2 (5), H2 (7), J2 (10), and K2 (12), concomitantly isolated from the root of Glycyrrhiza uralensis Fischer (Tohoku-kanzo in Japanese), have been determined on the basis of chemical and physicochemical evidence. During the process, a facile chemical conversion method from olean-12-ene or olean-12-en-11-one oligoglycosides to an olean-11,13-diene oligoglycoside, has been found.

KEYWORDS Glycyrrhiza uralensis; Glycyrrhizae Radix; licorice-saponin F3; licorice-saponin G2; licorice-saponin H2; licorice-saponin J2; licorice-saponin K2; olean-11,13-diene oligoglycoside

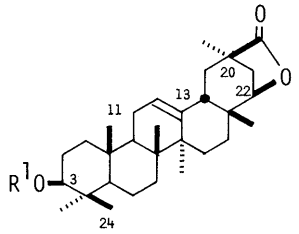
In our continuing chemical characterization studies of crude drug processing,[1] we have initiated a chemical study of botanically identified licorice roots of various origins. Recently, we isolated ten oleanene-triterpene oligoglycosides and glycyrrhizin (4) from the root of Glycyrrhiza uralensis Fischer (Leguminosae)[Tohoku-kanzo (東北甘草) in Japanese] and elucidated the chemical structures of five of them named licorice-saponins A3, B2 (9), C2 (11), D3 (3), and E2.[2] Afterwards, we found two sweet oligoglycosides, apioglycyrrhizin and araboglycyrrhizin, from the root of G. inflata [Shinkyo-kanzo (新疆甘草) in Japanese].[3] In this paper, we describe the structure elucidation of licorice-saponins F3 (1), G2 (5), H2 (7), J2 (10),[4] and K2 (12),[4] which were isolated together with licorice-saponins A3, B2, C2, D3, and E2 from the root of G. uralensis previously.[2,5] This paper also presents a facile chemical conversion method from an olean-12-ene or an olean-12-en-11-one oligoglycoside to an olean-11,13-diene oligoglycoside (i.e. 11 or 12).

Licorice-saponin F3 (1), mp 215-217 °C, $[\alpha]_D^{20}$ -20 °(MeOH), $C_{48}H_{72}O_{19}$,[6] IR (KBr, cm^{-1}) : 3400, 1760, 1720, ^1H NMR (500 MHz, pyridine-d_5, δ) : 5.36 (d, J = 7.6 Hz, 1'-H), 5.68 (d, J = 6.9 Hz, 1"-H), 6.10 (br.s, 1'"-H), gave deoxoglabrolide (2),[7] methyl glucuronide, and methyl rhamnoside, upon methanolysis. Ethereal CH_2N_2 treatment of 1 provided dimethyl ester 1a, mp 207-209 °C, $C_{50}H_{76}O_{19}$, IR (cm^{-1}) : 3420, 1745, ^1H NMR (δ) : 4.92 (d, J = 7.2 Hz, 1'-H), 5.67 (d, J = 7.3 Hz, 1"-H), 6.20 (br.s, 1'"-H), positive FAB MS (m/z) : 1003 $(M+Na)^+$.

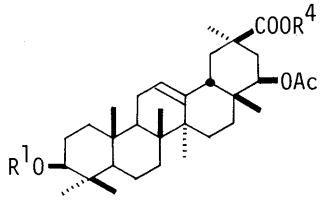
Methylation (CH_3I-DMSO-NaH) and subsequent $NaBH_4$ reduction of 1a followed by methanolysis, liberated methyl 3,4-di-O-methylglucopyranoside (a) and methyl 2,3,4-tri-O-methylrhamnopyranoside (b) in a 2:1 ratio. Comparison of ^{13}C NMR data for 1a (Table I) with those for D3 trimethyl ester (3a), led us to expect that licorice-saponins F3 (1) and D3 (3) have an identical trisaccharide moiety while F3 has a 30,22-lactone moiety in place of the 22β-OAc and 20β-COOH residues in D3. The expectation was verified by deacetylation (5% aq. KOH) of 3 and subsequent treatment with Dowex 50W x 8 (H^+) to furnish 1. Thus, the structure of licorice-saponin F3 (1) has been clarified as shown.

On ethereal CH_2N_2 treatment, licorice-saponin G2 (5), mp 229-230 °C, $[\alpha]_D^{20}$ +34 °(MeOH), $C_{42}H_{62}O_{17}$, UV (MeOH, nm, ε) : 249 (10600), IR (cm^{-1}) : 3350, 1720, 1648, ^1H NMR (δ) : 5.34 (d, J = 7.7 Hz, 1'-H), 5.59 (d, J = 6.7 Hz, 1"-H), ^{13}C NMR (125 MHz, pyridine-d_5, δc) : 63.1 (24-C), 104.4, 104.9 (1', 1"-C), positive FAB MS (m/z) : 877 $(M+K)^+$, 861 $(M+Na)^+$, 839 $(M+H)^+$, gave trimethyl ester 5a, mp 176-178 °C, $C_{45}H_{68}O_{17}$, IR (cm^{-1}) : 3367, 1720, 1648, ^1H NMR (δ) : 4.94 (d, J = 7.9 Hz, 1'-H), 5.56 (d, J = 7.6 Hz, 1"-H).

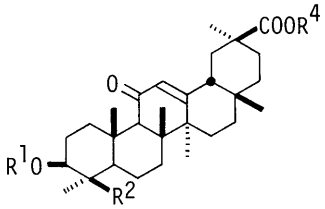
Methanolysis of 5a provided methyl 24-hydroxyglycyrrhetinate (6a)[8] and methyl glucuronide, while methanolysis, after $NaBH_4$ reduction and subsequent complete methylation of 5a, liberated methyl 2,3,4,6-tetra-O-methylglucopyranoside (c) and methyl 3,4,6-tri-O-methylglucopyranoside (d) in a 1:1 ratio. Based on these findings and examination of ^{13}C NMR data for 5a (Table I), the structure of licorice-saponin G2 has



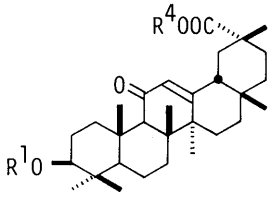
1 : R^1= Sr (licorice-saponin F3)
1a : R^1= SrMe
2 : R^1= H (deoxoglabrolide)



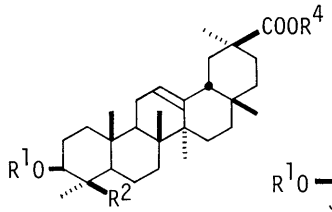
3 : R^1= Sr, R^4= H (licorice-saponin D3)
3a : R^1= SrMe, R^4= CH_3



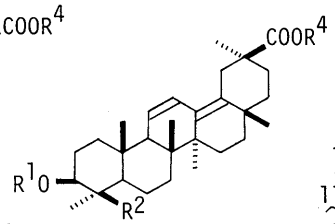
4 : R^1= S, R^2= CH_3, R^4= H (glycyrrhizin)
5 : R^1= S, R^2= CH_2OH, R^4= H (licorice-saponin G2)
5a : R^1= SMe, R^2= CH_2OH, R^4= CH_3
6a : R^1= H, R^2= CH_2OH, R^4= CH_3



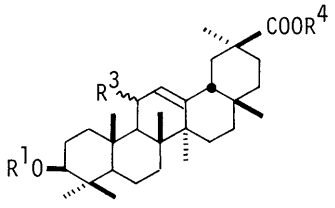
7 : R^1= S, R^4= H (licorice-saponin H2)
7a : R^1= SMe, R^4= CH_3
8 : R^1= R^4= H (liquiritic acid)



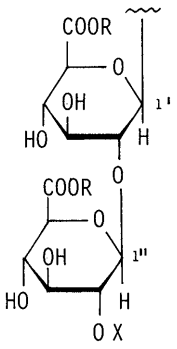
9 : R^1= S, R^2= CH_3, R^4= H (licorice-saponin B2)
9a : R^1= SMe, R^2= R^4= CH_3
10 : R^1= S, R^2= CH_2OH, R^4= H (licorice-saponin J2)
10a : R^1= SMe, R^2= CH_2OH, R^4= CH_3



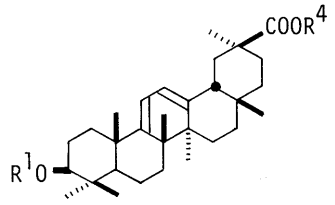
11 : R^1= S, R^2= CH_3, R^4= H (licorice-saponin C2)
11a : R^1= SMe, R^2= R^4= CH_3
12 : R^1= S, R^2= CH_2OH, R^4= H (licorice-saponin K2)
12a : R^1= SMe, R^2= CH_2OH, R^4= CH_3



13 : R^1= SMe, R^3= α-OAc & α-OCH_3, R^4= CH_3
14 : R^1= S, R^3= β-OH, R^4= H



S : R = X = H
SMe : R = CH_3, X = H
Sr : R = H, X = α-L-rham(py).
SrMe : R = CH_3, X = α-L-rham(py).



15 : R^1= S, R^4= H
15a : R^1= SMe, R^4= CH_3

Table I. ^{13}C NMR Data for 1a, 5a, 7a, 10a, and 12a (at 22.5 MHz in pyridine d_5, δc)

		1a	5a	7a	10a	12a
Sapogenol moiety	C-3	89.9	89.9	89.2	89.8	89.8
	C-11	47.5	198.9	199.0	47.5	125.3
	C-12	124.8	128.6	128.6	122.2	126.4
	C-13	140.6	168.7	168.4	144.3	135.2
	C-18	47.5	48.6	46.3	48.2	135.2
	C-22	84.3	38.2	37.1	36.2	36.2
	C-24	16.3	16.3	16.2	62.8	62.4
	C-29	28.0	28.0	178.1	28.1	28.1
	C-30	180.1	176.5	19.3	177.0	178.2
3-O-β-D-Glucurono-pyranosyl moiety	C-1'	104.7	104.1	104.9	104.0	104.0
	C-2'	79.1	81.9	84.3	81.1	80.8
	C-3'	76.3[a]	75.2	76.4[a]	76.3[a]	76.6
	C-4'	72.2[b]	72.2	72.5[b]	72.1	72.1
	C-5'	77.9[c]	77.2	77.3[c]	77.4[b]	77.7[a]
	C-6'	170.1[d]	169.6	170.1[d]	169.6	169.8
2'-O-β-D-Glucurono-pyranosyl moiety	C-1"	102.4	105.5	106.8	104.7	104.5
	C-2"	78.2	76.5[a]	76.6[a]	76.6[a]	76.6
	C-3"	76.6[a]	76.8[a]	77.4[c]	77.0[b]	77.0[a]
	C-4"	72.8[b]	72.2	72.9[b]	72.1	72.1
	C-5"	77.6[c]	77.2	77.6[c]	77.4[b]	77.4[a]
	C-6"	169.8[d]	169.6	170.2[d]	169.6	169.8
2"-O-α-D-Rhamno-pyranosyl moiety	C-1‴	101.6				
	C-2‴	71.8				
	C-3‴	72.8				
	C-4‴	73.9				
	C-5‴	69.1				
	C-6‴	18.5				

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

been determined as 5.

Licorice-saponin H2 (7), mp 209-210 °C, $[\alpha]_D^{25}$ +31 °(MeOH), $C_{42}H_{62}O_{16}$, UV (MeOH, nm, ε) : 248 (10700), IR (cm^{-1}) : 3400, 1725, 1650, ^1H NMR (δ) : 4.97 (d, J = 7.6 Hz, 1'-H), 5.35 (d, J = 7.6 Hz, 1"-H), ^{13}C NMR (δc) : 105.0 (1'-C), 106.8 (1"-C), 128.8 (12-C), 169.0 (13-C), 180.6 (29-C), 199.4 (11-C), positive FAB MS (m/z) : 861 $(M+K)^+$, 845 $(M+Na)^+$, liberated liquiritic acid (8) [9] and methyl glucuronide upon methanolysis.

Ethereal CH_2N_2 treatment of 7 afforded trimethyl ester 7a, mp 169 °C, $C_{45}H_{68}O_{16}$, IR (cm^{-1}) : 3362, 1722, 1654, ^1H NMR (δ) : 4.95 (d, J = 7.3 Hz, 1'-H), 5.33 (d, J = 7.7 Hz, 1"-H), positive FAB MS (m/z) : 887 $(M+Na)^+$,

which, upon methanolysis after $NaBH_4$ reduction and subsequent methylation, liberated methyl glycosides (c and d) as it did from 5a. These findings together with ^{13}C NMR data for 7a (Table I) led us to formulate licorice-saponin H2 as 7.

Licorice-saponin J2 (10), mp 263-265 °C, $[\alpha]_D^{25}$ +21 °(MeOH), $C_{42}H_{64}O_{16}$, IR (cm^{-1}) : 3405, 1729, 1615, ^1H NMR (δ) : 5.00 (d, J = 7.6 Hz, 1'-H), 5.65 (d, J = 7.0 Hz, 1"-H), was converted to trimethyl ester 10a, mp 198-199 °C, $C_{45}H_{70}O_{16}$, IR (cm^{-1}) : 3445, 1730, by CH_2N_2 methylation. These findings together with the examination of ^{13}C NMR data for 10a (Table I) led us to expect that J2 (10) was an 11-deoxo analog of licorice-saponin G2 (5). The expectation was verified by converting 5 in high yield to J2 (10) by reduction with zinc amalgam and HCl.

Methylation with CH_2N_2 of licorice-saponin K2 (12), mp 207-209 °C, $[\alpha]_D^{25}$ +28 °(MeOH), $C_{42}H_{62}O_{16}$, UV (MeOH, nm, ε) : 241 (13000), 249 (15000), 259 (9200), IR (cm^{-1}) : 3460, 1690, 1629, ^1H NMR (δ) : 5.04 (d, J = 7.6 Hz, 1'-H), 5.64 (d, J = 6.1 Hz, 1"-H), 5.54 (br.d, 11-H), 6.52 (br.d, 12-H), positive FAB MS (m/z) : 845 $(M+Na)^+$ afforded trimethyl ester 12a, mp 179-181 °C, $C_{45}H_{68}O_{16}$, IR (cm^{-1}) : 3424, 1731, 1621, positive FAB MS (m/z) : 903 $(M+K)^+$. These physicochemical properties and the examination of ^{13}C NMR data for 12a led us to presume the structure of licorice-saponin K2 (12) with a heteroannular 11,13-diene structure.[10]

To prove the structure 12, we carried out a conversion from known oligoglycoside. Thus, licorice-saponin B2 trimethyl ester (9a) was subjected to constant-current electrolysis (Pt, 40 mA/cm^2, MeOH-AcONa)[11] to provide a mixture of 11α-OAc and 11α-OMe derivatives (13). Treatment of 13 with 1% aq. HCl furnished licorice-saponin C2 trimethyl ester (11a) in 61% yield from 9a. On the other hand, $NaBH_4$ reduction of glycyrrhizin (4) in EtOH-H_2O (1:1) at 90 °C yielded an 11β-hydroxy derivative 14, mp 208-210 °C, $C_{42}H_{64}O_{16}$, ^1H NMR (δ) : 4.45 (dd, J = 6.5, 6.5 Hz, 11α-H),[12] in 95% yield. Treatment of 14 with 2% aq. HCl-dioxane (1:1) yielded a mixture of a 9,12-homoannular diene analog 15 and licorice-saponin C2 (11),[13] whereas treatment of 14 in dioxane-H_2O (1:1) with heating provided 11 in an excellent yield (82% from 4). Finally, $NaBH_4$ reduction of licorice-saponin G2 (5) and subsequent treatment of the product with dioxane-H_2O as above furnished licorice-saponin K2 (12) in 76% yield, thus the structure 12 was substantiated.

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REFERENCES AND NOTES

1) a) I.Kitagawa and M.Yoshikawa, J.Traditional Sino-Japanese Medicine, Vol.6, No.4, 101 (1985); b) Idem, ibid., Vol.7, No.3, 55 (1986); c) I.Kitagawa, T.Taniyama, M.Yoshikawa, Y.Ikenishi, and Y.Nakagawa, Chem. Pharm.Bull., 37, 2961 (1989), and previous papers cited therein.
2) I.Kitagawa, J.L.Zhou, M.Sakagami, T.Taniyama, and M.Yoshikawa, Chem.Pharm.Bull., 36, 3710 (1988).
3) I.Kitagawa, M.Sakagami, F.Hashiuchi, J.L.Zhou, M.Yoshikawa, and J.Ren, Chem.Pharm.Bull., 37, 551 (1989).
4) The trivial names licorice-saponins I2 and J2 (obtained in 0.002% respectively from the root) in previous paper[2] were changed to licorice-saponins J2 and K2, respectively in this paper.
5) M.Yoshikawa, J.L.Zhou, M.Sakagami, F.Hashiuchi, and I.Kitagawa, presented at the 108th Annual Meeting of the Pharmaceutical Society of Japan, held in Hiroshima, Apr.4-6, 1988, Abstract Papers p.316.
6) The molecular composition of the compound given with the chemical formula was determined by elemental analysis and(or) high resolution mass-spectrometry.
7) L.Canonica, G.Russo, and A.Bonati, Gazz.Chim.Ital., 96, 772 (1966).
8) L.Canonica, B.Danieli, P.Manitto, G.Russo, and A.Bonati, Gazz.Chim.Ital., 97, 1359 (1967).
9) L.Canonica, G.Russo, and E.Bombardelli, Gazz.Chim.Ital., 96, 833 (1966).
10) a) S.Shibata, I.Kitagawa, and H.Fujimoto, Chem.Pharm.Bull., 14, 1023 (1966); b) M.Nose, S.Amagaya, T.Takeda, and Y.Ogihara, ibid., 37, 1293 (1989).
11) a) I.Kitagawa, T.Kamigauchi, H.Ohmori, and M.Yoshikawa, Chem.Pharm.Bull., 28, 3078 (1980); b) M. Yoshikawa, H.K.Wang, V.Tosirisuk, and I.Kitagawa, Chem.Pharm.Bull., 30, 3057 (1982).
12) S.Shibata, K.Takahashi, S.Yano, M.Harada, H,Saito, Y.Tamura, A.Kumagai, K.Hirabayashi, M.Yamamoto, and N.Nagata, Chem.Pharm.Bull., 35, 1910 (1987).
13) The separation of 11 and 15 was effected after CH_2N_2 methylation to provide 11a and 15a, mp 180 °C, $C_{45}H_{68}O_{15}$, IR (cm^{-1}) : 3440, 1727, 1660, ^1H NMR (pyridine-d_5 + D_2O, δ) : 5.30, 5.31 (both d, J = 7.3 Hz, 11,12-H), ^{13}C NMR (22.5 MHz, pyridine-d_5, δc) : 154.9, 115.7, 125.5, 146.0 (9,11,12,13-C).

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