APIOGLYCYRRHIZIN AND ARABOGLYCYRRHIZIN, TWO NEW SWEET OLEANENE-TYPE TRITERPENE OLIGOGLYCOSIDES FROM THE ROOT OF GLYCYRRHIZA INFLATA

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Two new sweet oleanene-type triterpene oligoglycosides, named apioglycyrrhizin and araboglycyrrhizin, were isolated from the air-dried root of Glycyrrhiza inflata [Chinese Glycyrrhizae Radix, Shinkyo-kanzo in Japanese], and their structures have been determined on the basis of chemical and physicochemical evidence.

KEYWORDS Glycyrrhiza inflata; Glycyrrhizae Radix; apioglycyrrhizin; araboglycyrrhizin; oleanene-type triterpene oligoglycoside sweet taste; diazomethane methylation unusual; D-apiofuranoside ^{13}C NMR

In Japan, Glycyrrhizae Radix (licorice, the root of Glycyrrhiza sp.) has been used not only as an important ingredient in the prescriptions of Chinese traditional medicine but also as a sweetening in various cases. Glycyrrhizin (3), one of the bioactive constituents of Glycyrrhizae Radix, has also been used as a food additive for its sweetness. During the course of chemical studies on the bioactive constituents of naturally occurring drug materials,[1] we have recently isolated ten triterpene-oligoglycosides from Chinese Glycyrrhizae Radix [Tohoku-kanzo in Japanese], the air-dried root of Glycyrrhiza uralensis Fischer, and reported the structure elucidation of five of them, named licorice-saponins A3, B2, C2, D3, and E2.[2] In a continuing study, we have compared the chemical constituents of Glycyrrhizae Radix of various origins. This paper deals with the structure elucidation of two new sweet triterpene-oligoglycosides, apioglycyrrhizin (5) and araboglycyrrhizin (7), which were isolated from the air-dried root of Glycyrrhiza inflata Batal (Leguminosae) [Chinese Glycyrrhizae Radix, Shinkyo-kanzo (新疆甘草) in Japanese] together with glycyrrhizin (3), licorice-saponins A3,[2] G2,[3] and H2.[3,4]

The MeOH extract of the root was partitioned into an AcOEt-H_2O mixture and the H_2O-soluble portion was first subjected to reversed-phase silica gel column chromatography (Chromatorex ODS, 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 (Shim-pack PREP-ODS, CH_3CN-1% aq.AcOH), furnished apioglycyrrhizin (5, 0.32%),[5] mp 193-195°C, $[\alpha]_D^{25}$ +43° (MeOH), $C_{41}H_{62}O_{14}\cdot H_2O$,[6] UV λ_{max}^{MeOH} nm (ε): 249 (9200), IR ν_{max}^{KBr} cm^{-1}: 3360, 2914, 1716, 1650, and araboglycyrrhizin (7, 0.14%), mp 225-230°C, $[\alpha]_D^{25}$ +31° (MeOH), $C_{41}H_{62}O_{14}\cdot H_2O$, UV λ_{max}^{MeOH} nm (ε): 249 (9300), IR ν_{max}^{KBr} cm^{-1}: 3360, 2925, 1711, 1658, together with glycyrrhizin (3, 1.50%), licorice-saponins A3 (0.05%),[2] G2 (0.09%),[3] H2 (0.15%),[3] and known flavonoid glycosides [liquiritin (0.18%), liquiritin-apioside (0.60%), licuraside (0.54%)].

Complete methanolysis of apioglycyrrhizin (5) with 9% HCl-MeOH furnished methyl glucuronide, methyl apioside and glycyrrhetic acid (1), whereas partial methanolysis with the same reagent and subsequent alkaline hydrolysis with 10% aq.K_2CO_3 provided the prosapogenol 2.[7] The detailed ^1H NMR decoupling experiments (500 MHz, pyridine-d_5) of 5 resulted in the following assignments (J in Hz) for the oligosaccharide part: δ 4.18 (dd, J=8,9, 2'-H), 4.25 (br s, 5"-H_2), 4.28 (dd, J=9,9, 3'-H), 4.37, 4.69 (both d, J=9, 4"-H_2), 4.44 (dd, J=9,10, 4'-H), 4.51 (d, J=10, 5'-H), 4.90 (br s, 2"-H), 4.96 (d, J=8, 1'-H), 6.38 (br s, 1"-H). Treatment of 5 with diazomethane in MeOH at r.t. for 1 h yielded a dimethyl ester (5a), mp 219-220°C, $[\alpha]_D^{25}$ +37° (MeOH), $C_{43}H_{66}O_{14}\cdot 2H_2O$, UV λ_{max}^{MeOH} nm (ε): 249 (9630), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 2900, 1727, 1651. On the other hand, treatment of 5 with excess diazomethane in MeOH at r.t. for 24 h afforded a dimethyl ester 5a (45%) with concomitant formation of its 2"-O-methyl derivative (5b, 30%)[8] and 3"-O-methyl derivative (5c, 15%).[9]

To reproduce these unusual O-methylation of the alcoholic hydroxyl with diazomethane in MeOH, glycyrrhizin (3) was treated with excess diazomethane to provide a trimethyl ester (3a, 38%), the 2"-O-methyl derivative (3b, 25%),[10] and the 3"-O-methyl derivative (3c, 29%).[10] Thus, it has been shown that the treatment of glucuronide saponins such as 3 and 5 with excess diazomethane in MeOH yields 2"- and 3"-O-methyl derivatives as minor products.[11]

Treatment of 5a with $NaBH_4$ in MeOH furnished 6, mp 179-182°C, $[\alpha]_D^{25}$ +41° (MeOH), $C_{42}H_{66}O_{13}\cdot 2H_2O$, UV λ_{max}^{MeOH} nm (ε): 248 (9300), IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1727, 1651, which, on methanolysis, yielded methyl D-glucopyranoside,[12] methyl D-apiofuranoside,[12] and methyl glycyrrhetate (1a). Permethylation of 6 with CH_3I/DMSO/NaH[13] followed by methanolysis liberated methyl 2,3,5-tri-O-methylapiofuranoside and methyl 3,4,6-tri-O-methylglucopyranoside. Based on these findings and the ^{13}C NMR data comparisons (Table I), the structure of apioglycyrrhizin has been determined as 3-O-[β-D-apiofuranosyl(1→2)-β-D-glucuronopyranosyl] glycyrrhetic acid (5), in which the anomeric configuration at the D-apiofuranoside linkage has been substantiated by the application of Klyne's rule[14] and by the comparisons of the ^{13}C NMR data for 5 with those for

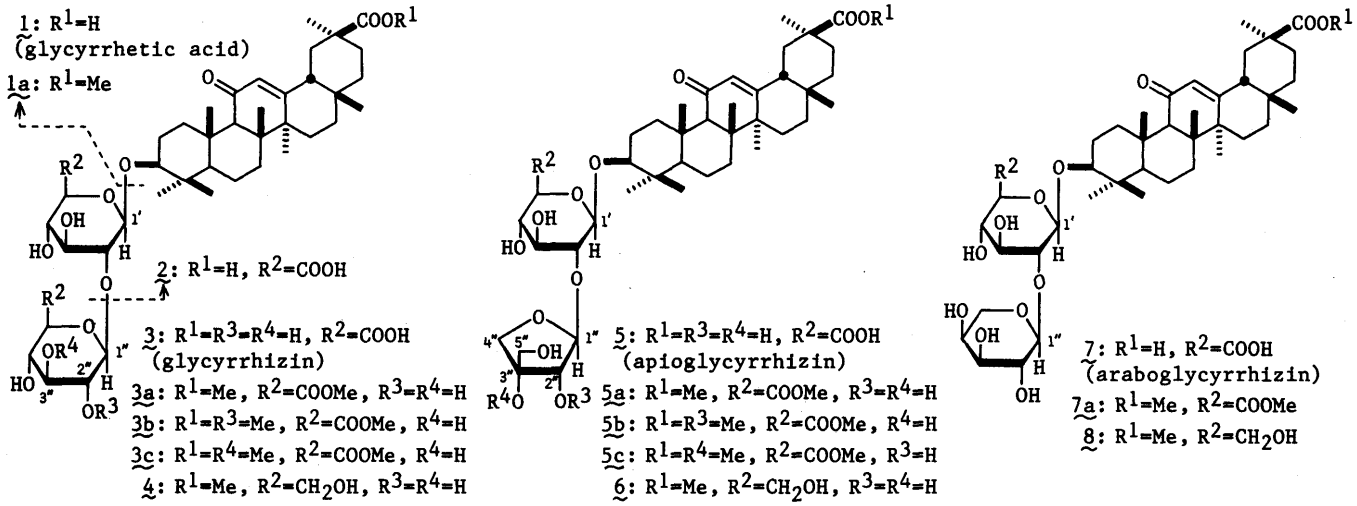

Table I. ^{13}C NMR Data for 5, 5a, 5b, 5c, 7, 7a, 3b and 3c (125 MHz, Pyridine-d_5, δ_C)

	5	5a	5b	5c	7	7a	3b	3c
C-3	88.8	89.1	89.2	89.2	88.9	89.0	89.5	89.2
C-11	199.1	199.4	199.4	199.4	199.4	199.3	199.2	199.3
C-12	128.4	128.7	128.8	128.7	128.6	128.6	128.5	128.6
C-13	169.3	168.9	169.0	169.0	169.5	168.9	168.8	169.7
C-30	178.8	176.8	176.8	176.8	179.1	176.7	176.6	176.8
30-OMe		51.6[a]	51.6[a]	51.6[a]		51.5[a]	51.5[a]	51.5[a]
C-1'	105.4	105.7	105.6	105.7	105.2	105.1	104.7	104.8
C-2'	79.4[a]	79.2[b]	80.8	79.3	83.4	83.1	85.0	84.1
C-3'	79.0[a]	78.1[c]	79.3	78.3	77.4	76.6[b]	76.6	77.2
C-4'	73.0	73.0	73.1	73.0	73.0	72.6	72.6[b]	72.3[b]
C-5'	77.8[b]	76.8	77.9	77.5	77.4	77.2[b]	78.0[c]	77.2[c]
C-6'	172.0	170.3	170.4	170.3	172.3	170.2	170.0	170.0
6'-OMe		51.9[a]	52.0[a]	51.9[a]		51.9[a]	51.8[a]	51.8[a]
C-1"	110.9	111.2	109.0	111.5	106.6	106.6	104.0	106.2
C-2"	77.6[b]	77.7[c]	87.2	76.8	73.6	73.5	80.6	75.7
C-3"	80.1[a]	80.4[b]	76.8	85.3	74.1	74.1	75.7	86.9
C-4"	75.2	75.5	75.6	71.7	69.2	68.9	72.4[b]	71.9[b]
C-5"	65.3	65.9	66.0	63.0	66.9	66.8	77.8[c]	76.4[c]
C-6"							169.6	169.7
2"-OMe			58.7				55.0	
3"-OMe				52.8[a]				60.8
6"-OMe							51.8[a]	52.0[a]

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

methyl α- and β-D-apiofuranosides.[15]

Methanolysis of araboglycyrrhizin (7) afforded methyl glucuronide, methyl arabinoside, and glycyrrhetic acid (1). Diazomethane methylation of 7 furnished 7a, a white powder, $[\alpha]_D^{25}$ +27° ($CHCl_3$), $C_{43}H_{66}O_{14}\cdot 2H_2O$, UV λ_{max}^{MeOH} nm (ε): 249 (11650), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 2900, 1722, 1641, ^1H NMR (500 MHz, pyridine-d_5): δ 5.86 (s, 12-H), 5.16 (d, J=7 Hz, 1"-H), 4.96 (d, J=8 Hz, 1'-H). Treatment of 7a with $NaBH_4$ and subsequent methanolysis of the reduction product (8), provided methyl D-glucopyranoside,[12] methyl L-arabinoside,[12] and 1a. Permethylation of 8 followed by methanolysis liberated methyl 2,3,4-tri-O-methylarabinopyranoside and methyl 3,4,6-tri-O-methylglucopyranoside. Finally, the detailed ^{13}C NMR examinations of 7 and 7a (Table I), have led to the formulation of araboglycyrrhizin as 3-O-[α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyl] glycyrrhetic acid (7).

From the HPLC examination of the oligoglycosidic constituents of various Glycyrrhizae Radix, we have found that apioglycyrrhizin (5) and araboglycyrrhizin (7) occur in several specimens of Shinkyo-kanzo. It is of interest here that apioglycyrrhizin (5) is about 2 times sweeter than glycyrrhizin (3), while the sweetness of araboglycyrrhizin (7) is comparable to 3.

REFERENCES AND NOTES

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2) I.Kitagawa, J.L.Zhou, M.Sakagami, T.Taniyama, and M.Yoshikawa, Chem.Pharm.Bull., 36, 3710 (1988).

3) We have hitherto isolated these licorice-saponins (G2 and H2) from the root of Glycyrrhiza uralensis. The structures of these oligoglycosides will be reported in our forthcoming paper.

4) 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.

5) The % yield was calculated from the air-dried root.

6) The molecular composition of the compound given with the chemical formula was determined by elemental analysis.

7) M.Kanaoka, S.Yano, H.Kato, and T.Nakada, Chem.Pharm.Bull., 34, 4978 (1986).

8) 5b, mp 135-139°C, $[\alpha]_D^{25}$ +41° ($CHCl_3$), $C_{44}H_{68}O_{14}\cdot 3H_2O$, UV λ_{max}^{MeOH} nm (ε): 249 (9700), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3381, 2934, 1726, 1650, ^1H NMR (500 MHz, pyridine-d_5): δ 6.38 (d, J=2 Hz, 1"-H), 5.87 (s, 12-H), 4.99 (d, J=8 Hz, 1'-H), 4.44 (d, J=2 Hz, 2"-H), 3.74, 3.71, 3.69 (3H each, s). Treatment of 5b with $NaBH_4$ followed by methanolysis gave methyl 2-O-methylapiofuranoside, methyl glucopyranoside, and 1a.

9) 5c, mp 133-135°C, $[\alpha]_D^{25}$ +25° ($CHCl_3$), $C_{44}H_{68}O_{14}\cdot 2H_2O$, UV λ_{max}^{MeOH} nm (ε): 249 (9700), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3390, 2926, 1725, 1650, ^1H NMR (500 MHz, pyridine-d_5): δ 6.26 (d, J=2 Hz, 1"-H), 5.85 (s, 12-H), 4.90 (d, J=8 Hz, 1'-H), 4.83 (d, J=2 Hz, 2"-H), 3.73, 3.71, 3.63 (3H each, s). Treatment of 5c with $NaBH_4$ followed by methanolysis gave methyl 3-O-methylapiofuranoside, methyl glucopyranoside, and 1a.

10) 3b, mp 240-245°C, $[\alpha]_D^{25}$ +40° ($CHCl_3$), $C_{46}H_{70}O_{16}\cdot 2H_2O$, UV λ_{max}^{MeOH} nm (ε): 249 (11800), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3390, 2935, 1724, 1655, ^1H NMR (500 MHz, pyridine-d_5): δ 5.86 (s, 12-H), 5.66 (d, J=8 Hz, 1"-H), 4.97 (d, J=8 Hz, 1'-H), 3.87, 3.79, 3.73, 3.70 (3H each, s). 3c, mp 240-245°C, $[\alpha]_D^{25}$ +46° ($CHCl_3$), $C_{46}H_{70}O_{16}\cdot 2H_2O$, UV λ_{max}^{MeOH} nm (ε): 249 (11200), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3447, 2930, 1726, 1660, ^1H NMR (500 MHz, pyridine-d_5): δ 5.86 (s, 12-H), 5.32 (d, J=8 Hz, 1"-H), 4.94 (d, J=8 Hz, 1'-H), 3.87, 3.83, 3.72, 3.70 (3H each, s). The structures 3b and 3c, were substantiated on the basis of their $NaBH_4$ treatment and subsequent methanolysis.

11) a) Partial methylations of some D-glucopyranosides with diazomethane are known. It was reported that the methylation reaction was much favored in the presence of a small amount of $SnCl_3$ which was presumably a contaminant in aged methanol stored in a can.[11b] However, in our experiments, freshly distilled MeOH was used. We have found that methyl D-glucopyranoside and 4 are not methylated under our reaction conditions. The scope and limitation of this methylation reaction is under investigation.

b) M.Aritomi and T.Kawasaki, Chem.Pharm.Bull., 18, 677 (1970).

12) Acidic hydrolysis of the methyl glycoside gave D-glucose [$[\alpha]_D^{23}$ +47° (H_2O)], D-apiose [$[\alpha]_D^{23}$ +9.1° (H_2O)], or L-arabinose [$[\alpha]_D^{23}$ +97° (H_2O)].

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14) a) W.Klyne, Biochem.J., 47, xli (1950); b) $[M]_D$(apioglycyrrhizin, 5)-$[M]_D$(2) = -150°; $[M]_D$(methyl α-D-apiofuranoside) = +221°,[14c] and $[M]_D$(methyl β-D-apiofuranoside) = -167°.[14c]

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15) ^{13}C NMR (125 MHz, pyridine-d_5, δ_C) data for methyl α-D-apiofuranoside: 104.5 (C-1), 75.2 (C-2), 77.7 (C-3), 73.4 (C-4), 65.5 (C-5), 55.0 (1-OCH_3) and for methyl β-D-apiofuranoside: 111.5 (C-1), 77.7 (C-2), 80.3 (C-3), 74.9 (C-4), 65.5 (C-5), 55.5 (1-OCH_3).

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