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 ¹³C 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-H₂O mixture and the H₂O-soluble portion was first subjected to reversed-phase silica gel column chromatography (Chromatorex ODS, H₂O-MeOH) to separate the oligoglycoside fraction. Repeated separation of the oligoglycoside fraction by ordinary-phase silica gel column chromatography (CHCl₃-MeOH-H₂O) and subsequent HPLC (Shim-pack PREP-ODS, CH₃CN-1% aq. AcOH), furnished apioglycyrrhizin (5, 0.32%),⁵⁾ mp 193-195°C, $[\alpha]_D^{25} + 43^\circ$ (MeOH), C₄₁H₆₂O₁₄·H₂O,⁶⁾ UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 249 (9200), IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3360, 2914, 1716, 1650, and araboglycyrrhizin (7, 0.14%), mp 225-230°C, $[\alpha]_D^{25} + 31^\circ$ (MeOH), C₄₁H₆₂O₁₄·H₂O, UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 249 (9300), IR $\nu_{\max}^{\text{KBr}}$ 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. K₂CO₃ provided the prosapogenol 2.⁷⁾ The detailed ¹H NMR decoupling experiments (500 MHz, pyridine-d₅) 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''-H₂), 4.28 (dd, J=9,9, 3'-H), 4.37, 4.69 (both d, J=9, 4''-H₂), 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, $[\alpha]_D^{25} + 37^\circ$ (MeOH), C₄₃H₆₆O₁₄·2H₂O, UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 249 (9630), IR $\nu_{\max}^{\text{CHCl}_3}$ 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 NaBH₄ in MeOH furnished 6, mp 179-182°C, [α]_D²⁵ +41° (MeOH), C₄₂H₆₆O₁₃·2H₂O, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 248 (9300), IR $\nu_{\text{max}}^{\text{KBr}}$ 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 CH₃I/DMSO/NaH¹³ 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 ¹³C NMR data comparisons (Table I), the structure of apioglycyrrhizin has been determined as 3-O- β -D-apiofuranosyl(1 $\rightarrow$ 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 rule¹⁴ and by the comparisons of the ¹³C NMR data for 5 with those for

Table I. ¹³C NMR Data for 5, 5a, 5b, 5c, 7, 7a, 3b and 3c (125 MHz, Pyridine-d₅, 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, $[\alpha]_D^{25} + 27^\circ$ (CHCl₃), C₄₃H₆₆O₁₄·2H₂O, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 249 (11650), IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3350, 2900, 1722, 1641, ¹H NMR (500 MHz, pyridine-d₅): 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 NaBH₄ 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 ¹³C NMR examinations of 7 and 7a (Table I), have led to the formulation of araboglycyrrhizin as 3-O-[α -L-arabinopyranosyl(1 $\rightarrow$ 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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- 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, $[\alpha]_D^{25} + 41^\circ$ (CHCl₃), C₄₄H₆₈O₁₄·3H₂O, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 249 (9700), IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3381, 2934, 1726, 1650, ¹H NMR (500 MHz, pyridine-d₅): 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 NaBH₄ followed by methanolysis gave methyl 2-O-methylapiofuranoside, methyl glucopyranoside, and 1a.
- 9) 5c, mp 133-135°C, $[\alpha]_D^{25} + 25^\circ$ (CHCl₃), C₄₄H₆₈O₁₄·2H₂O, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 249 (9700), IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3390, 2926, 1725, 1650, ¹H NMR (500 MHz, pyridine-d₅): 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 NaBH₄ followed by methanolysis gave methyl 3-O-methylapiofuranoside, methyl glucopyranoside, and 1a.
- 10) 3b, mp 240-245°C, $[\alpha]_D^{25} + 40^\circ$ (CHCl₃), C₄₆H₇₀O₁₆·2H₂O, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 249 (11800); IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3390, 2935, 1724, 1655, ¹H NMR (500 MHz, pyridine-d₅): 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, $[\alpha]_D^{25} + 46^\circ$ (CHCl₃), C₄₆H₇₀O₁₆·2H₂O, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 249 (11200), IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3447, 2930, 1726, 1660, ¹H NMR (500 MHz, pyridine-d₅): 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 NaBH₄ 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 SnCl₃ 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.
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- 12) Acidic hydrolysis of the methyl glycoside gave D-glucose [$[\alpha]_D^{23} + 47^\circ$ (H₂O)], D-apiose [$[\alpha]_D^{23} + 9.1^\circ$ (H₂O)], or L-arabinose [$[\alpha]_D^{23} + 97^\circ$ (H₂O)].
- 13) S.Hakomori, J.Biochem. (Tokyo), **55**, 205 (1964).
- 14) a) W.Klyne, Biochem.J., **47**, xli (1950); b) $[M]_D(\text{apio glycyrrhizin, 5}) - [M]_D(2) = -150^\circ$; $[M]_D(\text{methyl } \alpha\text{-D-apiofuranoside}) = +221^\circ$,^{14c)} and $[M]_D(\text{methyl } \beta\text{-D-apiofuranoside}) = -167^\circ$.^{14c)}
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- 15) ¹³C NMR (125 MHz, pyridine-d₅, 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-OCH₃) 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-OCH₃).

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