Phytochemistry, Vol. 24, No. 2, pp. 339–341, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

FLAVONOID GLYCOSIDES OF THE ROOTS OF *GLYCYRRHIZA URALENSIS*

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(*Revised received* 18 *July* 1984)

Key Word Index—*Glycyrrhiza uralensis*; Leguminosae; licorice roots; water extract; flavonoid glycosides; ononin; liquiritin; 4′-*O*-[β-D-apio-D-furanosyl-(1 → 2)-β-D-glucopyranosyl]liquiritigenin.

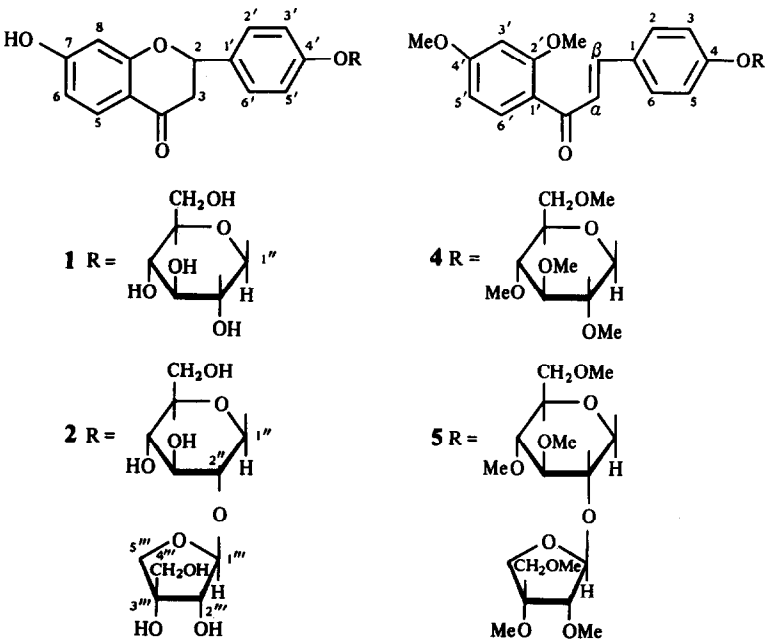
Abstract—The structure of a flavanone glycoside from the roots of *Glycyrrhiza uralensis* has been confirmed as 4′-*O*-[β-D-apio-D-furanosyl-(1 → 2)-β-D-glucopyranosyl]liquiritigenin. In addition, two known flavonoid glucosides, ononin (a minor component) and liquiritin (a major component), were isolated from the same extract.

INTRODUCTION

The roots of *Glycyrrhiza glabra*, *G. glabra* var. *glandulifera* and *G. uralensis* or licorice roots are widely used medicinally and for sweetening [1, 2]. Up to now, a number of triterpenoids and flavonoids have been characterized from these roots [2–4]. Since aqueous root extracts are used for medicinal purposes, the water-soluble chemical constituents of roots of *Glycyrrhiza uralensis* were examined, the results of which are described here.

RESULTS AND DISCUSSION

The isoflavone glucoside ononin was characterized [5] on the basis of the spectral evidence and the chemical correlation with authentic daidzin (see Experimental). This is the first report on the isolation of ononin from the roots of *G. uralensis* and also from licorice roots. The isolated liquiritin (**1**) had mp [6, 7] and optical rotation [7, 8] identical to published data. The UV, IR, FDMS, ^{1}H NMR and ^{13}C NMR spectra were consistent with this



3 R=H

structure. Acidic hydrolysis of **1** gave 1 mol each of glucose and liquiritigenin (**3**) [3, 6, 9].

The third root constituent (**2**), $C_{26}H_{30}O_{13}$, gave 1 mol each of glucose, apiose and liquiritigenin (**3**) [3, 6, 9] on acid hydrolysis. On mild acid hydrolysis (ion exchange resin), **2** gave **1** as a partial hydrolysate. In addition to this chemical evidence, the FDMS, UV, CD (*S*-configuration at C-2 in **2** [8]), ^{1}H NMR and ^{13}C NMR spectral data provide a gross structure, 4′-*O*-(apiosyl-β-D-glucopyranosyl)liquiritigenin for **2**. The β-oriented anomeric configuration of the inner D-glucopyranoside in **2** was also confirmed by the anomeric proton (1″-H) signal (δ4.99, *d*, J = 7.5 Hz) in the ^{1}H NMR spectrum. The ^{13}C NMR (100 MHz, DMSO-d_6) data of **2** were directly compared with those of **1** and authentic apiin [= 7-*O*-[β-D-apio-D-furanosyl-(1 → 2)-β-D-glucopyranosyl]apigenin] [10]. The chemical shifts of the disaccharide carbons of **2** were in good agreement with those of apiin [11, 12]. C-2″, C-1″ and C-3″ on the glucosyl moiety of **2** were respectively shifted by δ −2.7 (downfield), 1.7 and 0.5 (upfield) compared with those of **1** (glycosylation shift). Thus **2** is clearly 4′-*O*-[apiofuranosyl-(1 → 2)-β-glucopyranosyl]liquiritigenin and appears to be identical to the flavanone glucoside recently reported in licorice roots by Yahara and Nishioka [13]. However, the structure of the disaccharide moiety was not fully defined by these authors, so that we now report on these matters here.

Conclusive proof on the presence of D-apio-D-furanoside in **2** and the exact (β) configuration of the glycosidic linkage of D-apio-D-furanoside were obtained from the following experiments. Methylation of **1** and **2** afforded the corresponding chalcone permethylates **4** and **5**, respectively. Methanolysis of **5** gave methyl 2,3,4-tri-*O*-methyl-β-apio-D-furanoside and methyl 3,4,6-tri-*O*-methylglucopyranoside, identical to those prepared from authentic apiin in a similar manner. The isolated apiose-permethylate showed an optical rotation of −66°, consistent with methyl 2,3,4-tri-*O*-methyl-β-D-apio-D-furanoside (−79°) [14] and hence the presence of D-apio-D-furanose in **2** is indicated. The anomeric configuration of the terminal D-apio-D-furanose in **2** was assigned as β on the basis of the difference in molecular rotation between **1** and **2** ($\Delta[M]_D$ −150°; lit. [15]: methyl α-D-apio-D-furanoside, $\Delta[M]_D$ +221°; methyl β-D-apio-D-furanoside, $\Delta[M]_D$ −167°) and that between **4** and **5** ($\Delta[M]_D$ −147°; lit. [14]: methyl 2,3,4-tri-*O*-methyl-α-D-apio-D-furanoside, $[M]_D$ +239°; the corresponding β-anomer, $[M]_D$ −163°). Thus **2** is finally defined as 4′-*O*-[β-D-apio-D-furanosyl-(1 → 2)-β-D-glucopyranosyl]liquiritigenin. Finally, it appears that ononin, liquiritin and **2** occur (TLC and HPLC) in the aqueous extracts of both *G. glabra* and *G. glabra* var. *glandulifera* roots, the identification of which was carried out by one of us [K.Y., unpublished results].

EXPERIMENTAL

General remarks. Mps are uncorr. FDMS using carbon emitters: accelerating voltage, 3 kV; emitter current, 15–29 mA; chamber temp., room temp.; MS: at 70 eV. ^{1}H NMR and ^{13}C NMR: respectively at 400 and 100 MHz using TMS as internal standard. CD spectrum and optical rotations: in MeOH. IR: KBr discs, unless otherwise noted. GC with FID: 2 m × 3 mm packed with 1.5% SE-52 on Chromosorb W. Silica gel for CC: Kieselgel 60 (Merck). TLC: pre-coated silica gel plates (Merck HF-254).

Plant material. The licorice roots used in this study were collected in the north-east of China and imported. The source plant was identified as *G. uralensis* by one of us (K.Y.).

Isolation of glycosides. The air-dried and crushed roots (10 g) were extracted with H_2O (300 ml) at 90–100° for 40 min. Evapn of the extract gave a syrup (3.8 g). The accumulated syrup (34 g) was treated with MeOH (200 ml) and the resulting ppt. was filtered off. The filtrate gave, after evapn of the MeOH, a brown syrupy residue (17.5 g). Most (17.0 g) of the residue was chromatographed over silica gel (400 g) with $CHCl_3$–MeOH–H_2O (7:3:1; lower phase) eluants. Ononin (0.06 g), liquiritin (**1**) (3.0 g) and **2** (1.2 g) were respectively eluted in this order.

Ononin, colourless crystals, mp 215–217°, $[\alpha]_D^{25}$ −58.9° (*c* 0.12). MS *m/z* (rel. int.): 430 $[M]^+$ (4), 268 $[M-162]^+$ (100); FDMS *m/z* (rel. int.): 430 $[M]^+$ (100), 268 (28); ^{1}H NMR (MeOH-d_4): δ3.35–3.60 (4H, *m*, 2″, 3″, 4″, 5″-Hs of glc), 3.71 (1H, *dd*, J = 12 and 5.9 Hz) and 3.93 (1H, *dd*, J = 12 and 2.2 Hz) (6″-H_2 of glc), 3.83 (3H, *s*, OMe), 5.11 (1H, *d*, J = 7.0 Hz, 1″-H of glc), 7.00 and 7.48 (2H each, A_2B_2q, J = 7.0 Hz, 2′, 3′, 5′, 6′-Hs), 7.22 (1H, *dd*, J = 9.0 and 2.0 Hz, 6-H), 7.25 (1H, *d*, J = 2.0 Hz, 8-H), 8.15 (1H, *d*, J = 9.0 Hz, 5-H), 8.23 (1H, *s*, 2-H). The mp and UV (MeOH) spectrum were identical to those reported for authentic ononin [5] and the MS, FDMS and ^{1}H NMR spectra were consistent with the ononin structure. The isolated natural sample was in agreement (mixed mp, IR, ^{1}H NMR and TLC) with synthetic ononin prepared from authentic daidzin by methylation.

Liquiritin (**1**), colourless crystals, mp 209–211° (ref. [6], mp 212°; ref. [7], mp 212–213°), $[\alpha]_D^{25}$ −56.0° (*c* 0.21) (ref. [7], −54.7°; ref. [8], −70.5° (MeOH)). UV λ_{max}^{MeOH} nm (log ε): 313 (3.75), 275 (4.00); +NaOMe: 335, 255; +$AlCl_3$: 313, 275; IR ν_{max} cm^{-1}: 3300 (OH), 1640 (CO), 1600; FDMS *m/z* (rel. int.): 418 $[M]^+$ (100), 256 $[M-162]^+$ (82), 255 (95); ^{1}H NMR (MeOH-d_4): δ2.73 (1H, *dd*, J = 17.0 and 2.9 Hz) and 3.04 (1H, *dd*, J = 17.0 and 12.9 Hz) (3-H_2), 3.35–3.50 [4H, *m*, (2″–5″)-H_4 of glc], 3.70 (1H, *dd*, J = 12.0 and 5.4 Hz) and 3.90 (1H, *dd*, J = 12.0 and 2.2 Hz) (6″-H_2 of glc), 4.94 (1H, *d*, J = 7.5 Hz, 1″-H of glc), 5.45 (1H, *dd*, J = 12.9 and 2.9 Hz, 2-H), 6.36 (1H, *d*, J = 2.2 Hz, 8-H), 6.50 (1H, *dd*, J = 8.6 and 2.2 Hz, 6-H), 7.11 (2H, B_2 part of A_2B_2q, J = 8.8 Hz, 3′,5′-H_2) and 7.44 (2H, A_2 part of A_2B_2q, J = 8.8 Hz, 2′,6′-H_2), 7.72 (1H, *d*, J = 8.6 Hz, 5-H); ^{13}C NMR (DMSO-d_6): δ78.59 (C-2), 43.14 (C-3), 189.68 (C-4), 128.29 (C-5), 110.50 (C-6), 164.58 (C-7), 102.56 (C-8), 162.97 (C-9), 113.56 (C-10), 132.37 (C-1′), 127.82 (C-2′), 116.26 (C-3′), 157.44 (C-4′), 116.26 (C-5′), 127.82 (C-6′), 100.47 (C-1″ of glc), 73.22 (C-2″ of glc), 76.64 (C-3″ of glc), 69.81 (C-4″ of glc), 77.02 (C-5″ of glc), 60.76 (C-6″ of glc). Isolated **1** (250 mg) was hydrolysed in 10% H_2SO_4–EtOH (1:1; 40 ml) to give 1 mol each of glucose (by TLC and PC) and liquiritigenin (**3**) (see below).

2, an amorphous powder, $[\alpha]_D^{25}$ −69.9° (*c* 0.26) ([13], $[\alpha]_D^{19}$ −79.6°). UV λ_{max}^{MeOH} nm (log ε): essentially same as in ref. [13]; +NaOMe: 335, 255; +$AlCl_3$: 313, 275; FDMS *m/z* (rel. int.): 573 $[M+Na]^+$ (10), 551 $[M+H]^+$ (15), 418 $[M-132]^+$ (28), 256 $[M-132-162]^+$ (100); IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 1650 (CO), 1600; ^{1}H NMR (400 MHz, MeOH-d_4): δ2.75 (1H, *dd*, J = 17.0 and 2.9 Hz) and 3.04 (1H, *dd*, J = 17.0 and 12.9 Hz) (3-H_2), 3.35–3.65 [4H, *m*, (2″–5″)-H_4 of glc], 3.54 (2H, *s*, 4‴-H_2 of api), 3.70 (1H, *dd*, J = 12.0 and 5.4 Hz) and 3.89 (1H, *dd*, J = 12.0 and 2.2 Hz) (6″-H_2 of glc), 3.80 and 4.66 (1H each, ABq, J = 7.5 Hz, 5‴-H_2 of api), 3.95 (1H, *d*, J = 1.6 Hz, 2‴-H of api), 4.99 (1H, *d*, J = 7.5 Hz, 1″-H of glc), 5.45 (1H, *dd*, J = 12.9 and 2.9 Hz, 2-H), 5.47 (1H, *d*, J = 1.6 Hz, 1‴-H of api), 6.37 (1H, *d*, J = 2.2 Hz, 8-H), 6.50 (1H, *dd*, J = 8.6 and 2.2 Hz, 6-H), 7.12 (2H, B_2 part of A_2B_2q, J = 8.8 Hz, 3′,5′-H_2) and 7.44 (2H, A_2 part of A_2B_2q, J = 8.8 Hz, 2′,6′-H_2), 7.73 (1H, *d*, J = 8.6 Hz, 5-H) and see ref. [13] (100 MHz, DMSO-d_6); ^{13}C NMR (100 MHz, DMSO-d_6):

δ78.59 (C-2), 43.11 (C-3), 189.68 (C-4), 128.21 (C-5), 110.68 (C-6), 165.03 (C-7), 102.56 (C-8), 163.00 (C-9), 113.31 (C-10), 132.41 (C-1'), 127.90 (C-2'), 116.08 (C-3'), 157.28 (C-4'), 116.08 (C-5'), 127.90 (C-6'), 98.77 (C-1'' of glc), 75.93 (C-2''), 76.14 (C-3''), 69.99 (C-4''), 76.95 (C-5''), 60.64 (C-6''), 108.70 (C-1''' of api), 76.84 (C-2'''), 79.19 (C-3'''), 64.30 (C-4'''), 73.91 (C-5''') and see ref. [13] (25.05 MHz); CD: essentially same to that in ref. [13] (Found: C, 55.12; H, 5.78. Calc. for $C_{26}H_{30}O_{13} \cdot H_2O$: C, 54.93; H, 5.67%). ^{13}C NMR (100 MHz, DMSO-d_6) of the disaccharide part of apiin: δ98.27 (C-1'' of glc), 75.98 (C-2''), 76.13 (C-3''), 69.86 (C-4''), 77.02 (C-5''), 60.58 (C-6''), 108.75 (C-1''' of api), 76.75 (C-2'''), 79.15 (C-3'''), 64.20 (C-4'''), 73.92 (C-5''') and see refs. [11, 12].

Acidic hydrolysis of **2**. A soln of **2** (24 mg) in 10% H_2SO_4–EtOH (1:1; 4 ml) was refluxed for 3 hr. The mixture was poured into ice-water and extracted with Et_2O. After evapn of the solvent, the residue was recrystallized from EtOH–Et_2O to afford an aglycone (5 mg), colourless crystals, mp 203° (ref. [3], mp 207°; ref. [6], mp 205°). UV: see refs. [6, 9]; MS: see ref. [6]; IR ν_{max} cm^{-1}: 3200 (OH), 1640 (CO), 1600; ^1H NMR (MeOH-d_4): δ2.70 (1H, *dd*, *J* = 17.0 and 2.9 Hz) and 3.05 (1H, *dd*, *J* = 17.0 and 12.9 Hz) (3-H_2), 5.37 (1H, *dd*, *J* = 12.9 and 2.9 Hz, 2-H), 6.35 (1H, *d*, *J* = 2.2 Hz, 8-H), 6.50 (1H, *dd*, *J* = 8.6 and 2.2 Hz, 6-H), 6.81 (2H, B_2 of A_2B_2q, *J* = 8.8 Hz, 3',5'-H_2) and 7.33 (2H, A_2 of A_2B_2q, *J* = 8.8 Hz, 2',6'-H_2), 7.73 (1H, *d*, *J* = 8.6 Hz, 5-H); ^{13}C NMR (100 MHz, DMSO-d_6): essentially as in ref. [13] (25.05 MHz). The aglycone was identified by comparison of the mp, UV and MS data with those [3, 6, 9] published for liquiritigenin (**3**). The ^1H NMR and ^{13}C NMR data were consistent with structure **3**. The aq. layer was, after usual work-up, subjected to TLC ($CHCl_3$–MeOH–H_2O, 14:6:1) and PC (*n*-BuOH–EtOH–H_2O, 52:32:16; detected with aniline hydrogen phthalate) to demonstrate the presence of apiose and glucose [TLC: R_f 0.47 (api), 0.17 (glc); PC: R_f 0.43 (api), 0.24 (glc)]. The apiose standard used here was prepared from authentic apiin.

Partial hydrolysis of **2**. To a soln of **2** (650 mg) in MeOH (40 ml), ion exchange resin (Dowex 50W × 8) (*ca* 10 g) was added and the mixture was stirred for 6 hr at room temp. After removal of the resin by filtration, the filtrate was evapd to dryness. The residue was separated by silica gel column chromatography to give a partial hydrolysate (360 mg) identical (mmp, IR, MS and ^1H NMR) to liquiritin (**1**), together with the recovered starting material (**2**) (190 mg).

Methylation of **1** *and* **2**. Glucoside **1** (300 mg) was repeatedly (twice) methylated with MeI and $HCON(Me)_2$ in the presence of Ag_2O to afford the corresponding chalcone permethylate (**4**) (150 mg), pale yellow crystals, mp 122–123°, $[\alpha]_D^{25}$ −43.2° ($CHCl_3$; *c* 0.53). UV λ_{max}^{MeOH} nm (log ε): 337 (4.26); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1645 (CO), 1605; MS *m/z* (rel. int.): 502 $[M]^+$ (14), 284 (18), 218 (100); ^1H NMR ($CDCl_3$): δ3.20–3.70 [6H, *m*, (2''–6'')-Hs of glc], 3.39, 3.56, 3.65, 3.66, 3.87, 3.90 (3H each, all *s*, 6 × OMe), 4.88 (1H, *d*, *J* = 7.3 Hz, 1''-H of glc), 6.50 (1H, *d*, *J* = 2.2 Hz, 3'-H), 6.56 (1H, *dd*, *J* = 8.6 and 2.2 Hz, 5'-H), 7.03, 7.53 (2H each, A_2B_2q, *J* = 8.6 Hz, 2,3,5,6-Hs), 7.40 (1H, *d*, *J* = 15.8 Hz, α-H), 7.63 (1H, *d*, *J* = 15.8 Hz, β-H), 7.74 (1H, *d*, *J* = 8.6 Hz, 6'-H) (Found: C, 64.15; H, 6.57. $C_{27}H_{34}O_9$ requires: C, 64.53; H, 6.82%).

In a similar manner, **2** (300 mg) gave the corresponding chalcone permethylate (**5**) (200 mg), a pale yellow oil, $[\alpha]_D^{15}$ −54.9° ($CHCl_3$; *c* 0.30). UV λ_{max}^{MeOH} nm (log ε): 337 (4.28); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1645 (CO), 1600; FDMS *m/z* (rel. int.): 662 $[M]^+$ (100); ^1H NMR ($CDCl_3$): δ3.20–3.90 [9H, *m*, (2''–6'')-Hs of glc and 2''', 4'''-Hs of api], 3.22, 3.39, 3.43, 3.51, 3.56, 3.66, 3.87, 3.90 (3H each, all *s*, 8 × OMe), 4.05, 4.07 (2H, AB*q*, *J* = 10.4 Hz, 5'''-H_2 of api), 4.89 (1H, *d*, *J* = 7.8 Hz, 1''-H of glc), 5.47 (1H, *d*, *J* = 1.7 Hz, 1'''-H of api), 6.50 (1H, *d*, *J* = 2.3 Hz, 3'-H), 6.56 (1H, *dd*, *J* = 8.6 and 2.3 Hz, 5'-H), 7.03, 7.53 (2H each, A_2B_2q, *J* = 8.6 Hz, 2,3,5,6-Hs), 7.40 (1H, *d*, *J* = 15.8 Hz, α-H), 7.63 (1H, *d*, *J* = 15.8 Hz, β-H), 7.74 (1H, *d*, *J* = 8.6 Hz, 6'-H).

Methanolysis of **5**. A soln of **5** (5 mg) in dry 5% HCl–MeOH (1 ml) was heated under reflux for 4 hr. The soln was cooled and neutralized with Ag_2CO_3. After the usual work-up, two methylated monosaccharides were obtained and respectively identified by GLC (column temp., 155°; N_2 flow rate, 40 ml/min) and TLC (*n*-hexane–Me_2CO, 1:1) to Me 2,3,4-tri-*O*-Me-β-apio-D-furanoside (GLC: R_t 1'48''; TLC: R_f 0.61) and Me 3,4,6-tri-*O*-Me-glucopyranoside (GLC: R_t 4'05'' (sh), 4'13''; TLC: R_f 0.38), prepared from authentic apiin via methylation and methanolysis. The isolated apiose-permethylate showed an optical rotation of −66° ($CHCl_3$; *c* 0.12) and therefore, it is assigned to Me 2,3,4-tri-*O*-Me-β-D-apio-D-furanoside (−79° in $CHCl_3$ [14]).

Acknowledgements—We thank Professor J. Shoji, Showa University, Japan, and Dr. T. Tani, Kinki University, Japan, for generous gifts of authentic apiin and methyl 2,3,4-tri-*O*-methyl-β-D-apio-D-furanoside and of authentic daidzin, respectively.

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