Note

Synthesis of Glycyphyllin

Sachiko ESAKI, Manami ABE,
Naoko SHIBA and Shintaro KAMIYA

School of Food and Nutritional Sciences,
University of Shizuoka,
Yada 395, Shizuoka City 422, Japan

Received January 16, 1990

Dihydrochalcone phlorizin (phloretin 2'-*O*- β -D-glucoside, **1**) is the characteristic bitter flavonoid of apple trees and is known to have various physiological actions on animals and plants.¹⁾ Glycyphyllin (**2**), the sweet principle isolated from a sweet-tasting Australian shrub (*Smilax glycyphylla*) by Rennie,²⁾ is known to have the structure of phloretin 2'-*O*- α -L-rhamnoside.^{2,3)} Until now, however, **2** has not yet been synthesized.

In this paper, an attempt is reported of the synthesis of **2** and a related compound, phloretin 2'-*O*- α -D-mannoside (**3**), in order to compare the biological actions of **2** and **3** with **1**.

In the synthesis of **1**, Zemplén *et al.*⁴⁾ used 4-*O*-benzoylphloracetophenone 2-*O*-(tetra-*O*-acetyl- β -D-glucopyranoside) as the synthetic intermediate, which could be prepared by treating 4-*O*-benzoylphloracetophenone (**4**)⁵⁾ with tetra-*O*-acetyl- α -D-glucopyranosyl bromide in the presence of KOH in acetone. This method, however, cannot be applied to synthesize the α -L-rhamnoside (**5**), because the reaction of 1,2-*trans*-acylglycosyl halide with phenols usually tends to form orthoesters.⁶⁾

We describe here a convenient preparation of **5** by the nitromethane-mercuric cyanide procedure.⁷⁾ Reaction under reflux of **5** with tri-*O*-acetyl- α -L-rhamnopyranosyl chloride (**6**, 2 equiv.)⁸⁾ in the presence of an excess of mercuric cyanide in nitromethane gave a mixture of **5** and its β -anomer (**7**), both of which had the same *R*_f value from a silica gel TLC in various solvent systems. Deacylation of the mixture gave **8** and its β -anomer (**9**), which could successfully be separated on a silica gel column. The anomeric configurations of **8** and **9** were confirmed by C-13 NMR spectral data⁹⁾ (¹*J*_{Cl'-H1'} of **8** and **9** were 170 Hz and 156 Hz, respectively) and also by comparing their [α]_D²⁰ values (−53° and +31°). Coupling of **8** with *p*-hydroxybenzaldehyde in aq. KOH gave chalcone **10**, which was subsequently hydrogenated over 10% Pd-C to yield **2**.

Compound **3** was also prepared in a way similar to the synthesis of **2**. Reaction of **4** with tetra-*O*-acetyl- α -D-mannopyranosyl chloride (**11**)¹⁰⁾ gave coupling product **12**, which after deprotecting gave phloracetophenone 2-*O*- α -D-mannopyranoside (**13**). The anomeric configuration of

13 was determined as α -form by the C-13 NMR spectrum (¹*J*_{Cl'-H1'} = 168 Hz) and also by comparing its molecular rotation ([*M*]_D + 146.8°) with those of methyl α -D-mannoside ([*M*]_D + 152.5°) and methyl β -D-mannoside ([*M*]_D − 102.8°). Reaction of **12** with *p*-hydroxybenzaldehyde in aq. KOH gave chalcone **14**, which was hydrogenated to afford **3**.

The physiological activities of **2** and **3** on animals and plants will be reported elsewhere.

Experimental

Thin-layer chromatographic analyses were carried out as follows using silica gel plates: solvent A, benzene-ethyl acetate = 10:1; solvent B, ethyl acetate saturated with water; solvent C, benzene-ethyl acetate = 7:3; solvent D, ethyl acetate-methanol-water = 80:14:10. Two percent ferric chloride in ethanol or 5% sulfuric acid in ethanol was used as the spray reagent.

Phloracetophenone 2-O- α -L-rhamnopyranoside (8) and its β -anomer (9). A mixture of **4** (5.44 g), **6** (7.4 g) and mercuric cyanide (8.1 g) in dry nitromethane (500 ml) was boiled under reflux. After 2 hr, additional **6** (4.96 g) was added, and the reaction was continued overnight under the same conditions. The mixture was then evaporated to a syrup, and the residue was extracted with chloroform and filtered. The filtrate was successively washed with aq. 10% KI and water, and dried over MgSO₄. After filtration, the filtrate was evaporated and the residue was reextracted with chloroform to separate the undissolved **4** and filtered. The filtrate was evaporated, and the residue (17.6 g) was chromatographed on a silica gel column (530 g), eluting with benzene-ethyl acetate (10:1) to afford a mixture of **5** and its β -anomer **7** as an amorphous powder (4.1 g, 37.8%). *R*_f value 0.41 (A). This powder was dissolved in 0.1 N methanolic sodium methoxide (140 ml), and the solution was left at room temperature for 2 hr. After neutralizing with Amberlite IR-120 (H) resin, the filtrate was evaporated to a syrup, which was shown to be a mixture of two deprotected products (**8** and **9**) by TLC (*R*_f 0.35 and 0.27). The syrup (2.7 g) was chromatographed on a silica gel column (130 g), eluting with ethyl acetate saturated with water to give **8** (1.4 g, 22.5% based on **4**), and **9** (0.25 g, 4.0% based on **4**). **8**. Needles, *R*_f 0.35 (B), mp 209–211°C, [α]_D²⁰ − 53° (*c* = 1, CH₃OH). *Anal.* Found: C, 53.27; H, 5.90. *Calcd.* for C₁₄H₁₈O₈: C, 53.50; H, 5.73%. NMR δ _C (DMSO-*d*₆): 17.85 (q, CH₃); 32.77 (q, CH₃); 69.89 (d, C-5'); 70.03 (d, C-2'); 70.64 (d, C-3'); 71.61 (d, C-4'); 93.68 (d, C-3); 96.42 (d, C-5); 99.34 (d, C-1', *J* = 170 Hz); 104.90 (s, C-1); 160.08 (s, C-6); 164.63 (s, C-4); 165.76 (s, C-2); 201.85 (CO).

9. Needles, *R*_f 0.27 (B), mp 120–123°C, [α]_D²⁰ + 31° (*c* = 1, CH₃OH). *Anal.* Found: C, 50.50; H, 6.21. *Calcd.* for C₁₄H₁₈O₈·H₂O: C, 50.00; H, 5.95%. NMR δ _C (DMSO-*d*₆): 17.77 (q, CH₃); 33.00 (q, CH₃); 70.36 (d, C-5'); 71.57 (d, C-2'); 72.33 (d, C-3'); 72.89 (d, C-4'); 93.98 (d, C-3);

- 1 S = β -D-glucosyl
 2 S = α -L-rhamnosyl
 3 S = α -D-mannosyl

- 4 $R_1 = H$; $R_2 = C_6H_5CO$
 5 $R_1 = \text{tri-O-acetyl-}\alpha\text{-L-rhamnosyl}$; $R_2 = C_6H_5CO$
 7 $R_1 = \text{tri-O-acetyl-}\beta\text{-L-rhamnosyl}$; $R_2 = C_6H_5CO$
 8 $R_1 = \alpha\text{-L-rhamnosyl}$; $R_2 = H$
 9 $R_1 = \beta\text{-L-rhamnosyl}$; $R_2 = H$
 12 $R_1 = \text{tetra-O-acetyl-}\alpha\text{-D-mannosyl}$; $R_2 = C_6H_5CO$
 13 $R_1 = \alpha\text{-D-mannosyl}$; $R_2 = H$

- 10 S = α -L-rhamnosyl
 14 S = α -D-mannosyl

96.41 (d, C-5); 97.90 (d, C-1', $J=156$ Hz); 104.94 (s, C-1); 160.61 (s, C-6); 164.53 (s, C-4); 165.55 (s, C-2); 202.87 (CO).

2',4,4',6'-Tetrahydroxychalcone 2'-O- α -L-rhamnopyranoside (**10**). Compound **8** (1.17 g) was triturated with ethanol (1 ml), and at 0°C treated with cold 60% KOH solution (8 ml). The mixture was stirred for 10 min and then *p*-hydroxybenzaldehyde (0.52 g) was added. The mixture was stirred for 4 days at room temperature. The

reddish-brown solution was cooled to 0°C and slightly acidified with ice-cold 10% hydrochloric acid. After 30 min, the crystals deposited were collected by filtration, washed with water and dried. The dried crystals were washed with benzene and recrystallized from aq. methanol to give yellowish **10** (0.7 g, 41.4%) as needles. *R*_f 0.47 (B), $[\alpha]_D^{20} +64^\circ$ ($c=1$, CH₃OH). *Anal.* Found: C, 55.50; H, 5.72. Calcd. for C₂₁H₂₂O₉·2H₂O: C, 55.46; H, 5.26%. NMR δ_c (DMSO-*d*₆): 17.95 (CH₃); 69.89 (C-5''); 70.07 (C-2''); 70.83 (C-3''); 71.93 (C-4''); 94.54 (C-3''); 96.92

(C-5'); 99.55 (C-1'); 106.11 (C-1'); 116.15 (C-3, 5); 123.38 (C₆); 125.76 (C-1); 130.67 (C-2', 6'); 143.49 (C₂); 159.02 (C-4); 160.00 (C-6'); 163.87 (C-4'); 165.15 (C-2'); 191.76 (CO).

2',4,4',6'-Tetrahydroxydihydrochalcone (phloretin) 2'-O- α -L-rhamnopyranoside (glycyphyllin, 2). Chalcone **10** (1 g) was hydrogenated in methanol (50 ml) with 10% Pd-C (1 g) as the catalyst for 100 min at atmospheric pressure. After filtration, the filtrate was crystallized from aq. ethanol to give **2** (0.73 g, 70.6%) as colorless needles, *R*_f 0.41 (B), mp 122°C, $[\alpha]_D^{20}$ -32° ($c=1$, C₂H₅OH). Lit.²⁾ mp 115–120°C $[\alpha]_D^{20}$ -33° . *Anal.* Found: C, 56.3; H, 6.22. Calcd. for C₂₁H₂₄O₉·1½ H₂O: C, 56.37; H, 6.04%. The hexaacetate, mp 136°C (lit. 136°C). UV $\lambda_{\max}$ (EtOH) nm (ϵ): 287; $\lambda_{\max}$ (2 mm EtONa) nm (ϵ): 324 (lit. $\lambda_{\max}$ (EtOH) nm (ϵ): 287; $\lambda_{\max}$ (2 mm EtONa) nm (ϵ): 327). NMR δ_C (DMSO-*d*₆): 28.88 (CH₂); 44.71 (CH₂); 69.90 (C-5'); 70.09 (C-2'); 70.93 (C-3'); 71.84 (C-4'); 94.28 (C-3'); 96.83 (C-5'); 99.86 (C-1'); 105.16 (C-1'); 115.14 (C-3, 5); 129.01 (C-2, 6); 131.15 (C-1); 155.25 (C-4); 159.71 (C-6'); 164.90 (C-4'); 165.12 (C-2'); 203.41 (CO).

4-O-Benzoylphloracetophenone 2-O-(2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside) (12). A mixture of **4** (5.44 g), **11** (8.8 g) and mercuric cyanide (8.1 g) in dry nitromethane (500 ml) was refluxed with stirring. After 2 hr, additional **11** (5.86 g) was added, and the reaction was continued overnight under the same conditions. The mixture was treated in a similar manner to that used for the preparation of **5**. The resulting syrup (19 g) was chromatographed on a silica gel column (570 g), eluting with benzene-ethyl acetate (10:1) to give **12** as a syrup (3.51 g, 29.2%), *R*_f 0.69 (C), $[\alpha]_D^{20}$ $+23^\circ$ ($c=1$, CHCl₃). *Anal.* Found: C, 57.50; H, 5.08. Calcd. for C₂₉H₃₀O₁₄: C, 57.80; H, 4.98%. NMR δ_C (DMSO-*d*₆): 20.18, 20.29, 20.35, 20.42 (each q, CH₃); 32.63 (q, CH₃); 61.62 (t, C-6'); 64.85 (d, C-4'); 68.07 (d, C-3'); 68.35 (d, C-2'); 69.22 (d, C-5'); 95.51 (d, C-1', $J=175$ Hz); 100.07 (d, C-3); 104.74 (d, C-5); 113.77 (s, C-1); 128.40 (s, C-1'); 128.89 (d, C-3', 5'); 129.67 (d, C-2', 6'); 134.14 (d, C-4'); 153.99 (s, C-6); 155.32 (s, C-4); 159.77 (s, C-2); 163.69 (s, COO), 169.28, 169.35, 169.49, 169.66 (each s, COO); 201.94 (CO).

Phloracetophenone 2-O- α -D-mannopyranoside (13). A solution of **12** (500 mg) in 0.1 N methanolic sodium methoxide (10 ml) was refluxed for 6 hr. The solution was then neutralized with Amberlite IR (H+) resin and filtered. The filtrate was evaporated, and the residue, after washing with benzene, was crystallized from aq. methanol to afford **13** (0.17 g, 60.7%) as needles, *R*_f 0.1 (B), mp 232°C, $[\alpha]_D^{20}$ $+56^\circ$ ($c=0.25$, CH₃OH). *Anal.* Found: C, 49.50; H, 6.15. Calcd. for C₁₄H₁₈O₉·1½ H₂O: C, 49.50; H, 5.60%. NMR δ_C (DMSO-*d*₆): 32.77 (CH₃); 60.86 (C-6'); 66.49 (C-4');

69.69 (C-3'); 70.87 (C-2'); 75.35 (C-5'); 94.06 (C-3); 96.44 (C-5); 99.55 (C-1', $J=168$ Hz); 104.90 (C-1); 160.20 (C-6); 164.64 (C-4); 165.64 (C-2); 201.07 (CO).

2,4,4',6'-Tetrahydroxychalcone 2'-O- α -D-mannopyranoside (14). Compound **12** (3.46 g) was dissolved in a mixture of aq. 60% KOH and ethanol (3 ml). To this, *p*-hydroxybenzaldehyde (760 mg) was added and the mixture was stirred for 4 days at room temperature. The reaction mixture was treated in a similar manner to that already described, and the crystals deposited from the solution were collected and recrystallized from aq. ethanol to give **14** as needles (1.06 g, 40.8%), *R*_f 0.57 (D), mp 210–212°C, $[\alpha]_D^{20}$ -20° ($c=1$, CH₃OH). *Anal.* Found: C, 56.08; H, 5.59. Calcd. for C₂₁H₂₂O₁₀·H₂O: C, 55.75; H, 5.31%. NMR δ_C (DMSO-*d*₆): 60.98 (C-6'); 66.85 (C-4'); 69.80 (C-3'); 71.14 (C-2'); 75.40 (C-5'); 94.99 (C-3'); 97.03 (C-5'); 99.79 (C-1'); 106.14 (C-1'); 116.21 (C-3, 5); 123.38 (C₆); 125.79 (C-1); 130.75 (C-2, 6); 143.55 (C₂); 159.27 (C-4); 160.03 (C-6'); 164.00 (C-4'); 165.17 (C-2'); 191.79 (CO).

2',4,4',6'-Tetrahydroxydihydrochalcone (phloretin) 2'-O- α -D-mannopyranoside (3). Compound **14** (1.35 g) in methanol (250 ml) was hydrogenated with Pd-C (10%, 1.35 g) for 1 hr and then treated as already described to give **3** as an amorphous powder, *R*_f 0.55 (D), $[\alpha]_D^{20}$ $+33^\circ$ ($c=1$, CH₃OH). *Anal.* Found: C, 56.60; H, 6.10. Calcd. for C₂₁H₂₄O₁₀·1½ H₂O: C, 56.62; H, 5.51%. NMR δ_C (DMSO-*d*₆): 28.86 (CH₂); 44.70 (CH₂); 60.92 (C-6'); 66.70 (C-4'); 69.75 (C-3'); 71.14 (C-2'); 75.38 (C-5'); 94.65 (C-3'); 96.82 (C-5'); 99.81 (C-1'); 105.21 (C-1'); 115.13 (C-3, 5); 129.01 (C-2, 6); 131.12 (C-1); 155.24 (C-4); 159.81 (C-6'); 164.83 (C-4'); 165.00 (C-2); 203.42 (CO).

References

- 1) G. Stenlid, *Phys. Plantarum*, **21**, 882 (1968).
- 2) E. H. Rennie, *J. Chem. Soc.*, **1886**, 857.
- 3) A. H. Williams, *Phytochemistry*, **6**, 1583 (1967).
- 4) G. Zemplén and R. Bognár, *Chem. Ber.*, **75**, 1040 (1942).
- 5) F. W. Canter, F. H. Curd and A. Robinson, *J. Chem. Soc.*, **1931**, 1245.
- 6) H. S. Isbell and H. L. Frush, *J. Res. Nat. Bur. Stand.*, **43**, 161 (1931).
- 7) T. Yamazaki, J. Yoshioka, D. A. Jeanloz and R. W. Jeanloz, *Carbohydr. Res.*, **89**, 342 (1981).
- 8) H. Ohle, W. Marecek and W. Bourjaw, *Chem. Ber.*, **62**, 833 (1929).
- 9) R. Kasai, M. Okihara, J. Asakawa, K. Mizutani and O. Tanaka, *Tetrahedron*, **35**, 1421 (1979).
- 10) M.-C. Courtin-Duchateau and A. Veyrieres, *Carbohydr. Res.*, **65**, 23 (1978).