6-C-α-L-Rhamnopyranosylapigenin 7-O-β-D-glucopyranoside (Isofurcatain 7-O-β-D-glucoside), a New Flavone Glycoside from *Metzgeria furcata*

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Metzgeria furcata, Metzgeriales, Hepaticae, 6-C-α-L-rhamnopyranosylapigenin (isofurcatain) 7-O-β-D-glucopyranoside, ^{13}C-NMR

The isolation of 6-C-α-L-rhamnopyranosylapigenin (isofurcatain) 7-O-β-D-glucopyranoside, from *Metzgeria furcata* var. *ulvula* is described together with its identification. This is a new natural product, as also is isofurcatain.

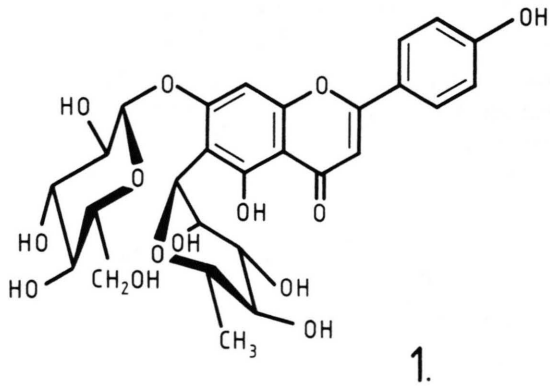
Introduction

Previous work on the flavonoid content of liverworts in the family Metzgeriaceae (comprising two genera, *Metzgeria* and *Apometzgeria*) has established the predominant presence of flavone di-C-glycosides of the aglycone types, apigenin, luteolin, tricetin and their methyl ethers [1 – 4]. New flavonoids isolated include a range of tricin, tricetin and apometzgerin (tricetin 3′,4′-di-O-methyl ether) di-C-glycosides [1, 2], and 2^{G}-O-ferulylisoschaftoside [3]. We report here the isolation and characterization of a new flavone glycoside from *Metzgeria furcata* var. *uvula*.

Results and Discussion

The 80% aqueous methanol extract of air-dried *M. furcata* plant material yielded, after chromatography on cellulose, a flavone glycoside, **1**, which crystallized from MeOH/H_2O as pale yellow needles (31 mg), m.p. 223 – 225 °C. Chromatographic and UV-visible absorption data suggested that the compound was a flavone glycoside of the apigenin (5,7,4′-trihydroxyflavone) type with a substituted 7-hydroxyl group [5]. Hydrolysis with trifluoroacetic acid (TFA) yielded glucose which was identified by cochromatography (TLC) with authentic glucose, together with an apigenin glycoside, m.p. 218 – 220 °C. This glycoside was shown to possess a free 7-hydroxyl group by UV-visible spectroscopy and did not yield an aglycone on further acid treatment. Such behaviour is consistent with that of an apigenin C-glycoside and the high chromatographic mobility in BAW indicated [6] that it was a mono-C-glycoside.

Mass spectrometry of the permethyl (PM) ethers of the original glycoside and the C-glycoside revealed molecular ions at m/z 704 and 500 respectively. The difference between these molecular ions confirms the presence of only one hydrolysable glucose unit in the original glycoside, and the molecular ion of the C-glycoside defines the C-linked sugar as a deoxyhexose. That this deoxyhexose is linked to the flavone at the C-6 position is evidenced by the high intensity of the M^+-31 ion in the MS of both the



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original glycoside and the C-glycoside [7, 8], and by the low field position (111.9 ppm) of C-6 in the ^{13}C-NMR [9] of **1**.

In the ^{13}C-NMR spectrum of the C-glycoside the chemical shifts of the sugar carbons were found to match closely with those reported [9] for the C-linked α-L-rhamnopyranosyl moiety in violanthin (6-C-β-D-glucopyranosyl-8-C-α-L-rhamnopyranosylapigenin [6]). This rhamnosyl formulation for the C-glycoside was confirmed by TLC co-chromatography of its permethyl ether with the permethyl ether of authentic (synthetic) 6-C-α-L-rhamnopranosylapigenin.

The 7-O-linked glucose is considered to be β-linked to the apigenin 6-C-rhamnoside since in the ^{1}H-NMR spectrum of the original glycoside, the glucose H-1 appears as a doublet ($J = 9.8$ Hz) centred at 5.26 ppm, *cf.* [5], [10]. Furthermore, in the ^{13}C-NMR spectrum, the pattern of sugar carbon signals associated with the O-linked glucose closely resembles that observed for saponarin (6-C-β-D-glucopyranosylapigenin 7-O-β-D-glucopyranoside) [9] but not that reported for β-D-glucofuranosides [11], thus confirming the pyranosidic nature of the glucose. The structure of the new glycoside is therefore defined as 6-C-α-L-rhamnopyranosylapigenin 7-O-β-D-glucopyranoside, (**1**). It is the 7-O-β-D-glucopyranoside of another new natural product, 6-C-α-L-rhamnopyranosylapigenin, for which we propose the name isofurcatain. The *iso* prefix is necessary to indicate the C-6 linkage of the sugar [6], and the name furcatain is chosen since furcatin and metzgerin have been used previously for natural products. Until now 6-C-rhamnosylapigenin has been known only as a product of synthesis [6, 12].

An interesting feature of isofurcatain 7-O-glucoside is the apparent steric interference of the 6-C-α-linked rhamnose with the 7-O-linked glucose (see **1**). This is evidenced in the ^{13}C-NMR spectrum by the chemical shifts of the sugar carbons which are markedly different from those expected from a combination of the signals of the 7-O-glucose unit in apigenin 7-O-glucopyranoside [9] and the 6-C-rhamnosyl unit in isofurcatain. Such a phenomenon is not observed in the spectrum of saponarin [9] where presumably there is less interference due to the C-linked sugar being β-linked. Neither saponarin nor glycoside **1** is hydrolysed by β-glucosidase, however; suggesting that some steric crowding does occur even when the C-linked sugar is β-linked.

Experimental

Plant material. Metzgeria furcata (L.) Dum. var. *ulvula* Nees was collected in May 1979 from tree trunks near Interlaken (Zweilütschinen), Bernese Alps, Switzerland. Voucher specimens are deposited in the Herbarium of the Fachrichtung Botanik, Universität des Saarlandes, Saarbrücken.

Extraction and isolation. The air-dried plant material (30 g) was extracted as described previously [13]. Glycoside **1** was isolated by repeated column chromatography on cellulose (microcrystalline, Merck) with 3% HOAc and *n*-amylalcohol:H_2O:HOAc, 2:1:1 as solvents.

Thin-Layer chromatography. hR_f values for *glycoside* **1** and *isofurcatain*, respectively, (a) on cellulose (F 1440, Schleicher and Schüll): 50, 21 (15% HOAc); 76, 64 (40% HOAc); 66, 88 (*n*-BuOH:HOAc:H_2O, 4:1:5, upper phase); 74, 90 (*n*-BuOH:HOAc:H_2O, 100:27:73) and (b) on polyamide (Macherey and Nagel): 65, 30 (80% MeOH); 56, 12 (H_2O:MeCOEt:MeOH:acetylacetone, 13:3:3:1). *Glucose* was identified by TLC on cellulose with the solvents: EtOAc:Pyr:HOAc:H_2O, 36:36:7:21; *n*-BuOH-:Pyr:HOAc:EtOAc:H_2O, 50:20:10:25:20; EtOAc-:Pyr:H_2O:HOAc:propionic acid, 10:10:2:1:1; and on silica (Merck) with the solvents: $CHCl_3$:MeOH:H_2O, 64:36:8 and EtOAc:MeOH:HOAc:H_2O, 15:3:3:2. *PM ethers* were purified by prep. TLC on silica gel G 60 (Merck) with solvents : EtOAc; $CHCl_3$:EtOAc:Me_2CO, 5:1:4 and 5:4:1. Spray reagents used with flavone glycosides: Naturstoffreagenz A [14] and Benedicts reagent [15].

Hydrolysis. Glycoside **1** was hydrolysed completely in 4 h with 1 N aqueous TFA under reflux.

UV-visible spectroscopic data (nm). Glycoside **1**: λ_{max} (MeOH) 273, 332; (NaOMe) 276, 305 sh, 354 sh, 396 (inc. intensity); ($AlCl_3$ and $AlCl_3$/HCl) 279, 302, 351, 382 sh; (NaOAc) 272, 344 sh, 391; (NaOAc/H_3BO_3) 273, 337. *Isofurcatain:* λ_{max} (MeOH) 271, 334; (NaOMe) 278, 328, 395 (inc. intensity); ($AlCl_3$ and $AlCl_3$/HCl) 279, 305, 353, 384 sh; (NaOAc) 278, 299, 382; (NaOAc/H_3BO_3) 273, 342.

Mass spectroscopy. PM ethers were prepared (see [16]) and the spectra recorded as previously described [17, 18] using probe temperatures in the range 160–290 °C. *PM-Glycoside* **1** (*m/z* with relative intensities in brackets; alphabetic identification symbols relate to those used by Bouillant *et al.*, [8]):

704(42), M^+; 689(18), a_2(M) = M^+-15; 673 (100), b_3(M) = M^+-31; 659(1), c_1(M) = M^+-45; 657(7), c_3(M) = M^+-47; 631(7), g(M) = M^+-73; 629(8), g-2(M) = M^+-75; 571(10), h(M) = M^+-133; 559(12), i(M) = M^+-145; 486(41), AH = M^+-218; 471(38), a_2(AH) = AH-15; 455(71), b_3(AH) = AH-31; 441(11), c_1(AH) = AH-45; 439(22), c_3(AH) = AH-47; 423(82), b_3-MeOH(AH) = AH-63; 341(55), i(AH) = AH-145. *PM-Isofurcatain* (*m/z*, alphabetic identification symbols relate to those used by Bouillant *et al.*, [8]): 500(3), M^+; 486(3), a_1=M^+-14; 485(12), a_2=M^+-15; 471(4), b_1=M^+-29; 470(17), b_2=M^+-30; 469(62), b_3 = M^+-31; 453(14), c_3 =M^+-47; 427(18), gd = M^+-73; 367(14), hd = M^+-133; 355(100), id = M^+-145, 341(38), jd = M^+-159; 325(34), kd = M^+-175; 311(31), ld = M^+-189.

^{1}*H-NMR spectroscopy* (ppm downfield from TMS in DMSO-d_6/D_2O at 30 °C). *Glycoside* **1:** 7.94 (d, J = 8.6 Hz) H-2′,6′; 6.93 (d, J = ca. 8.6 Hz); 6.98, 6.83 (singlets) H-3 and H-8; 5.28 (d, J = 9.8 Hz) glycose H-1; 4.85 (br. doublet, J = 6.2 Hz) rhamnose H-1; 4.5–3.0 (multiplets) sugar protons; 1.36 (d, J = ca. 7 Hz) rhamnose CH_3.

^{13}*C-NMR spectroscopy* (ppm downfield from TMS in DMSO-d_6 at 30 °C). *Glycoside* **1**: 182.1 (C-4), 164.1 (C-2), 163.3 (C-7), 161.3 (C-4′) 159.7 (C-5), 156.2 (C-9), 128.5 (C-2′,6′), 120.9 (C-1′), 116.0 (C-3′,5′), 111.9 (C-6), 105.1 (C-10), 103.2 (C-3), 102.3 (C-1, glu), 94.6 (C-8), sugar carbons at 77.4, 75.4, 73.6 (2 carbons), 72.2, 71.5, 69,7, 64.8, 62.1, 60.7 and 16.3 (CH_3, rha). *Isofurcatain:* 182.0 (C-4), 164.1 (C-2), 163.8 (C-7), 161.4 (C-4′), 158.2 (C-5), 156.6 (C-9), 128.7 (C-2′, 6′), 121.3 (C-1′), 116.2 (C-3′,5′), 108.0 (C-6), 103.2 and 102.8 (C-3 and C-10), 95.1 (C-8), 77.3 (C-1″), 74.5 and 74.3 (C-2″ and C-3″), 72.2 and 72.0 (C-4″ and C-5″), 18.3 (C-6″).

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[1] R. Theodor, H. D. Zinsmeister, R. Mues, and K. R. Markham, Phytochemistry **19,** 1695 (1980).
[2] R. Theodor, K. R. Markham, R. Mues, and H. D. Zinsmeister, Phytochemistry **20,** 1457 (1981).
[3] R. Theodor, H. D. Zinsmeister, R. Mues, and K. R. Markham, Phytochemistry **20,** 1851 (1981).
[4] R. Theodor, H. D. Zinsmeister, R. Mues, and K. R. Markham, Phytochemistry, in press.
[5] T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer, New York 1970.
[6] J. Chopin and M.-L. Bouillant, in: The Flavonoids (J. B. Harborne, T. J. Mabry, and H. Mabry, eds), p. 632, Chapmann and Hall, London 1975.
[7] M.-L. Bouillant, A. Besset, J. Favre-Bonvin, and J. Chopin, Phytochemistry **17,** 527 (1978).
[8] M.-L. Bouillant, J. Favre-Bonvin, and J. Chopin, Phytochemistry **14,** 2267 (1975).
[9] K. R. Markham, V. M. Chari, and T. J. Mabry, in: Advances in Flavonoid Research, 1975–1980 (J. B. Harborne and T. J. Mabry, eds) Chapman and Hall, London 1982.
[10] W. E. Hillis and D. H. S. Horn, Australian J. Chem. **18,** 531 (1965).
[11] R. G. S. Ritchie, N. Cyr, B. Korsch, H. J. Koch, and A. S. Perlin, Canadian J. Chemistry **53,** 1424 (1975).
[12] J. Chopin and M. C. Biol, Comptes Rendus (C) **275,** 1435 (1972).
[13] R. Mues and H. D. Zinsmeister, Phytochemistry **15,** 1757 (1976).
[14] R. Neu, Naturwissenschaften **44,** 181 (1957).
[15] H. Reznik and K. Egger, Z. Analyt. Chem. **183,** 196 (1961).
[16] K. R. Markham, Techniques of Flavonoid Identification, p. 63. Academic Press, London 1982.
[17] H. Schels, H. D. Zinsmeister, and K. Pfleger, Phytochemistry **16,** 1019 (1977).
[18] H. Wagner and O. Seligmann, Tetrahedron **29,** 3029 (1973).