Structure Determination of 6-C-β-D-glucopyranosyl-8-C-α-L-arabinopyranosyltricetin from *Radula complanata*

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The flavone di-C-glycoside earlier isolated from *Radula complanata* and tentatively identified as tricetin 6-C-β-D-glucopyranosyl-8-C-α-L-arabinopyranoside is now positively identified by ^{13}C- and ^{1}H-NMR spectroscopy. Although this compound is thought to have been isolated before, this is the first substantial support for its structure, previously assigned on the basis of MS evidence.

Tricetin di-C-glycosides are rare flavone glycosides, detected so far only in some liverwort species [1, 2]. Recently 5 different tricetin di-C-glycosides were isolated from the liverwort *Radula complanata* (L.) Dum. [3]. One of them, compound Rc-3, was tentatively identified as 6-C-β-D-glucopyranosyl-8-C-α-L-arabinopyranosyltricetin. Chromatographic and UV-visible data (see Experimental) defined the aglycone moiety as tricetin, acid treatment failed to give an aglycone and the R_f values and colour reactions were similar to those of known tricetin di-C-glycosides. Further, the mass spectrum of the permethylated (PM) glycoside is in accordance with a 6-C-hexosyl-8-C-pentosyl formulation, showing hexose-loss fragment peaks with higher intensity than the pentose-loss fragments [4]. Cochromatography with 6-C-glucopyranosyl-8-C-arabinopyranosyltricetin from *Metzgeria furcata* [2] indicated identity in all solvent systems on TLC, both for the original and for the PM-derivative. Other available tricetin di-C-glycosides were clearly separated.

The original structure assignment of the *M. furcata* reference compound itself however, was based only on UV-visible absorption and MS studies and cochromatography of the PM-derivative with PM 6-C-glucopyranosyl-8-C-arabinopyranosyltricin from *Apometzgeria pubescens* [5] gave identical spots on TLC in various solvents.

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The structure of Rc-3 has now been confirmed by ^{13}C- and high field ^{1}H-NMR spectroscopy (for data see Experimental and Tables I and II). In the ^{13}C-NMR spectrum, chemical shifts for the carbons of the flavonoid nucleus are in accord with those published for tricetin and isoaffinetin [6] (Table I). Differences are of course observed for C-6 and/or C-8 compared to tricetin and isoaffinetin (6-C-glucopyranosyltricetin). The chemical shift for the C-6 of Rc-3 is identical with those observed for C-6 of isoaffinetin and schaftoside (both C-linked β-D-glucopyranose residues), and C-8 also shows the downfield shift effect of C-glycosylation, appearing at 103.7 ppm (*cf.* schaftoside, 104.5 ppm). The ^{1}H-NMR spectrum (DMSO-d_6) also confirms the

Table I. Chemical shifts (ppm) for flavonoid nucleus carbons in the ^{13}C-NMR spectra of tricetin [a], iso-affinetin [a] and compound Rc-3.

	Tricetin	Isoaffinetin	Rc-3
C-4	181.6	182.0	181.6
C-2	164.2	164.1	163.6
C-7	164.2	163.7	159.8
C-5	161.2	160.9	159.8
C-9	157.5	156.5/157.2	(159.8?)
C-3′,5′	146.5	146.6	146.3
C-4′	137.9	138.1	138.2
C-1′	120.9	120.5	120.3
C-6	99.0	109.0	108.9
C-2′,6′	106.0	105.8	105.8
C-10	104.0	103.5	102.1
C-3	103.2	103.0	102.1
C-8	93.9	93.7	103.7

[a] Spectra and assignments from reference 6.

Table II. Chemical shifts (ppm) of sugar carbons in the ^{13}C-NMR spectra of schaftoside and Rc-3.

Atom Nr.	Glucose (Schaftoside)	Glucose (Rc-3)	Arabinose (Schaftoside)	Arabinose (Rc-3)
1	73.7 [a] 73.6 [b]	73.4	74.6 [a] 74.7 [b]	74.1
2	71.1 [a] 70.7 [b]	70.3	68.9 [a] 68.8 [b]	68.8
3	78.8 [a] 78.7 [b]	79.1	75.1 [a] 75.1 [b]	75.5
4	70.2 [a] 69.8 [b]	70.3	69.2 [a] 68.8 [b]	68.8
5	81.5 [a] 81.2 [b]	81.4	71.1 [a] 70.7 [b]	70.3
6	61.0 [a] 61.5 [b]	61.1	–	–

[a] Chemical shift listed in reference 6.
[b] Chemical shift listed in reference 7.

aglycone structure (see Experimental). Thus apart from sugar proton signals, the only signals present are those representing the 5-hydroxyl (δ13.81) H-2′,6′ (δ7.03) and H-3 (δ6.51).

The ^{13}C-NMR spectrum of Rc-3 in the sugar carbon region is almost identical with that of schaftoside (Table II) and proves the β-linkage and pyranose ring structure of the arabinose residue [6]. In contrast, β-linked L-arabinose (in neoschaftoside) has recently been shown to exhibit C-1 at 71.4 ppm [7], 2.7 ppm upfield from the arabinose C-1 position in Rc-3. In Rc-3, the α-linkage of the arabinose and the β-linkage of the glucose are also clearly defined in the ^{1}H-NMR spectrum (DMSO-d_6) in which the glucose H-1 (δ4.65) exhibits a coupling constant of 9.9 Hz and the arabinose H-1 (δ4.80) exhibits a coupling constant of 9.3 Hz. These constants are essentially the same as reported for schaftoside but differ from those of neoschaftoside [7].

Two glycosides possibly with the same structure as Rc-3 have been previously reported. The leafy liverwort *Plagiochila asplenioides* contains a 6-C-hexopyranosyl-8-C-pentopyranosyltricetin [8] and Markham and Porter [9] have reported that the primitive liverwort, *Takakia lepidozioides* accumulates a tricetin 6-C-hexoside-8-C-pentoside to which they tentatively assigned the 6-C-glucosyl-8-C-arabinoside structure. In neither of these cases however is there sufficient supporting evidence to permit confident assignment of the Rc-3 structure. There is no other report of this glycoside from any other natural source.

Experimental

Compound Rc-3 was isolated from H_2O-extracts of 49 g air-dried *Radula complanata*, collected from bark of trees near Interlaken, Switzerland. The compound was separated from other glycosides by CC on cellulose (Merck, microcrystalline) with 3 and 15% HOAc; on Sephadex LH-20 (Pharmacia) with MeOH and MeOH:H_2O (80:20) and by PC (Whatman 3 MM) with the solvents 3 and 15% HOAc and BAW (upper layer). The compound was crystallized from H_2O. 28 mg were obtained, m.p. >220 °C with decomposition. R_f-values (TLC, microcrystalline, cellulose, Schleicher and Schüll, F 1440, ready plates): TBA: 0.08; BAW: 0.12; 15% HOAc: 0.23. Fluorescence after fuming with NH_3: yellow; Fluorescence after spraying with NA (0.1% MeOH): orange-yellow. *UV-visible data:* MeOH: 354, 273 NaOMe: 422, 334 sh, 275 (dec.) $AlCl_3$: 419, 314 sh, 280 $AlCl_3$/HCl: 386, 366, 307, 280 NaOAc: 403, 330 sh, 283 NaOAc/H_3BO_3: 436 sh, 394, 272 nm *^{1}H-NMR data* (200 MHz; DMSO-d_6, δ): 13.81 (singlet), 5-OH; 7.03 (singlet) H-2′,6′; 6.51 (singlet) H-3; 4.89 (d, J = 9.3 Hz) H-1 arabinose; 4.65 (d, J = 9.9 Hz) H-1 glucose; 4.3 – 3.1 (multiplet) sugar protons. *^{1}H-NMR data* (80 MHz; TMS ether in $CDCl_3$, δ): 7.24 (2H, singlet) H-2′,6′; 7.02 (1H, singlet) H-3; 3–5 (13H, multiplet) sugar protons.

^{13}C-NMR data (200 MHz, DMSO-d_6, ppm): 181.6 (C-4), 163.6 br (C-2), 159.8 (C-5,7 and 9?), 146.3 (C-3′,5′), 138.2 (C-4′), 120.3 (C-1′), 108.9 (C-6), 105.8 (C-2′,6′), 103.7 (C-8), 102.1 br (C-3,10), 81.4 (G-5), 79.1 (G-3), 75.5 (A-3), 74.1 (A-1), 73.4 (G-1), 70.3 (G-2,4 A-5), 68.8 (A-2,4), 61.1 (G-6).

MS-data (PM-glycoside): M^+ = 764(23); $M^+ - 15$ = 749(31); $M^+ - 31$ = 733(100); $M^+ - 47$ = 717(12); $M^+ - 103$ = 661(17); $M^+ - 119$ = 645(9); $M^+ - 131$ = 633(23); $M^+ - 145$ = 619(9); $M^+ - 163$ = 601(48); $M^+ - 175$ = 589(59); $M^+ - 189$ = 575(16). (Values are given in m/z and in parentheses the % abundance relative to the base peak; base peak = peak of highest intensity above m/z = 150.)

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