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Chemical Studies on Bryophytes. 19. Application of ^{13}C NMR in Structural Elucidation of Flavonoid *C*-Glucosides from *Hedwigia ciliata*

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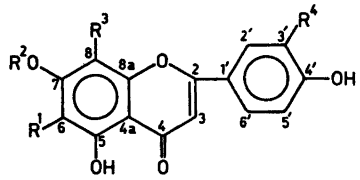
Two flavonoid *C*-glucosides have been isolated from the moss *Hedwigia ciliata*. By means of ^{13}C NMR studies of the two compounds and of a few known *C*-glucosides, the two *C*-glucosides have been identified as apigenin 6,8-di-*C*-β-D-glucopyranoside (vicenin-2, *1*) and luteolin 6,8-di-*C*-β-D-glucopyranoside (lucenin-2, *2*).

In previous communications the structures of two new luteolin tetraglycosides isolated from the moss *Hedwigia ciliata* were reported.[1,2] The present paper reports the identification of two di-*C*-glucosides (*1* and *2*).

UV spectral data of *1* and *2* indicate the aglycone moiety to be apigenin and luteolin, respectively.[3] This was confirmed by ^1H NMR spectroscopy of their TMS ethers.[3] The ^1H NMR spectra also indicate that both *1* and *2* are diglycosides. Upon acid treatment of *1* and *2* no aglycone or free sugar was formed suggesting that the two flavonoids are *C*-glycosides.



1, $R^1=R^3=$ β-D-G (= β-D-glucopyranosyl) and $R^2=R^4=H$; *2*, $R^1=R^3=$ β-D-G, $R^2=H$ and $R^4=OH$; *3*, $R^3=$ β-D-G and $R^1=R^2=R^4=H$; *4*, $R^1=$ β-D-G and $R^2=R^3=R^4=H$; *5*, $R^1=R^3=$ β-D-G and $R^3=R^4=H$; *6*, $R^3=$ β-D-G, $R^1=R^2=H$ and $R^4=OH$; *7*, $R^1=R^2=R^3=R^4=H$.

Since no additional spots appeared on the TLC-plates after 2-dimensional TLC, no Wessely-Moser-rearrangement occurred during acid treatment.[4] Thus *1*, as well as *2*, has the same sugar unit in the 6- and the 8-position.

To determine the structure of the sugar moieties of *1* and *2* ^{13}C NMR spectroscopy was applied. Several articles have been published on ^{13}C NMR spectroscopy of flavonoids,[5–13] but none have dealt with *C*-glycosides. The ^{13}C NMR data of *1*, *2* and related compounds are recorded in Tables 1 and 2. The ^{13}C NMR spectra are measured in DMSO-d_6, but since the luteolin di-*C*-glycoside *2*, like other luteolin di-*C*-glycosides, is very unstable in the presence of oxygen and decomposes or polymerizes in DMSO-d_6,[14] the ^{13}C NMR data of the TMS ether derivatives are also reported in Tables 1 and 2. The TMS ethers were chosen for the same reason as in ^1H NMR spectroscopy.[3] Other advantages are that the carbon signals of the TMS groups occur in a region of the ^{13}C NMR spectra (0 – 3 ppm) where no signals from the flavonoids are located, and that the same derivatives can be used for both ^1H NMR and ^{13}C NMR spectroscopy.

The assignments of the signals of the aglycone moieties in *1*, *2*, *3* (vitexin), *4* (isovitexin), *5* (saponarin) and *7* (apigenin) are based on those given in the literature.[5–13] The values reported for *2* in DMSO-d_6 are a little uncertain due to the fact that *2* decomposed during the recording of the spectrum. Comparison of the ^{13}C NMR spectra of these flavonoids before

Table 1. ^{13}C NMR shifts of the aglycone moieties of the isolated flavonoids and related compounds.[a]

Compound	2	3	4	5	6	7	8	8a	4a	1′	2′	3′	4′	5′	6′
1	163.9	102.6	182.2	155.0	107.6	161.4[c]	103.7	161.3[c]	105.3	121.5	129.0	115.9	158.7	115.9	129.0
1 TMS ether	161.4	106.7	178.1	156.2	119.2	158.2	111.6[c]	160.0	112.3[c]	125.0	128.5	120.7	157.2	120.7	128.5
2	165.8	104.1	183.8	157.2	108.1	163.9	105.0[c]	160.1	105.8[c]	123.6	115.2	146.4	150.5	117.5	121.3
2 TMS ether	161.3	106.7	178.1	156.2	119.2	157.3	111.6[c]	160.1	112.3[c]	125.4	119.2	147.0	150.0	121.0	121.4
3[b]	164.0	102.6	181.9	155.8	98.9	162.5	104.2	160.6[c]	104.2	121.8	128.5	116.0	160.9[c]	116.0	128.5
3 TMS ether	161.2	107.3	177.9	155.7	109.4	158.2[c]	111.9[d]	158.6[c]	111.6[d]	125.0	128.3	120.5	158.2[c]	120.5	128.3
4[b]	163.8	102.9	181.9	156.4	108.8	163.8	94.2	161.3[c]	103.5	121.2	128.4	116.3	160.6[c]	116.3	128.4
5	164.4	103.4	182.3	156.7	110.7	162.6	94.0	161.5[c]	105.2	121.1	128.8	116.3	159.6[c]	116.3	128.8
5 TMS ether	161.1	106.4	177.8	156.2	118.1	158.2	100.9	160.1	112.3	124.5	127.7	120.5	157.4	120.5	127.7
6 TMS ether	161.8	107.2	175.2	155.5	109.0	157.8	111.2[c]	159.8	112.9[c]	125.8	118.9	146.6	149.2	120.7	121.0
7	163.7[c]	102.9	181.7	157.3	99.0	164.1[c]	94.0	161.5[d]	103.8	121.3	128.4	116.0	161.1[d]	116.0	128.4
7 TMS ether	161.0	107.3	177.6	156.4	110.3	158.9[c]	101.9	159.5	112.3	124.8	127.7	120.4	158.1[c]	120.4	127.7
1 acetate	162.0	109.1	176.1	154.5[c]	120.3	155.5[c]	109.1	162.0	114.0	129.6	128.8	123.7	154.8[c]	123.7	128.8

[a] The TMS ethers were measured in $CDCl_3$, the acetate in acetone-d_6 and the others in DMSO-d_6. [b] Measured in 1 mm capillary tube. [c,d] Assignments bearing the same superscript in any one spectrum may be reversed.

and after trimethylsilylation makes it evident that the carbons with a TMS ether group are shifted upfield and the *ortho* and *para* carbons are shifted downfield. An upfield shift can also be observed for the carbonyl carbon at the 4-position and the carbon at the 2-position whereas the carbon at the 3-position is shifted downfield. The *C*-glycosylation of a flavonoid shifts the signal of the corresponding aglycone carbon downfield by 8.6 – 10.2 ppm and the *ortho* carbons upfield by 0.1 – 1.4 ppm. Other carbon signals are not significantly affected. The same changes can be seen in the TMS ethers.

A tentative assignment of the signals from the sugar moieties of *1* – *6* is shown in Table 2. The sugar moieties in the compounds *3* – *6* have earlier been identified as β-D-glucopyranosyl units. The values for carbon in the different compounds correspond well to each other, and there is no significant difference between the glucose signals whether the glucose moiety is attached in the 6- or in the 8-position, as can be seen in vitexin (*3*) and isovitexin (*4*). The upfield shift of the C-1 carbon in glucose, observed when comparing a flavonoid *C*-glucoside with an *O*-glucoside, is the same as that observed between phenyl-β-D-glucopyranoside and β-D-glucopyranosylbenzene (Table 3). The most surprising difference in the comparison of the flavonoid *C*-glucosides with the β-D-glucopyranosylbenzene is the value of the C-3 signal. This is shifted upfield by 3.7 – 6.4 ppm in the flavonoid *C*-glucosides compared to the same carbon in β-D-glucopyranosylbenzene. The reason for this might be the *ortho* hydroxyl groups in the flavonoid *C*-glucosides. Other workers have obtained similar values of a few flavonoid *C*-glycosides isolated from *Linum maritinum*.[15]

The ^{13}C NMR data of the acetylated *1* are also reported in Tables 1 and 2. The changes of the shifts in the aglycone moiety are the same as those reported earlier for acetylated phenols.[16] This is also true for the sugar units.[17,18] Tables 1 and 2 indicate the structures of *1* and *2* to be apigenin 6,8-di-*C*-β-D-glucopyranoside and luteolin 6,8-di-*C*-β-D-glucopyranoside, respectively.

Mass spectroscopy of permethylated (PM) and perdeuteriomethylated (PDM) *1* and *2* showed M^+ at *m/e* 748 and at *m/e* 781 for *1*

Table 2. ^{13}C NMR shifts of the sugar moieties of the isolated flavonoids and related compounds and a tentative assignment of the signals.[a]

Compound	C-1[c]	C-2	C-3[d]	C-4[d]	C-5[c]	C-6
1	78.0 78.9	74.1 74.9	72.0 73.4	69.2 70.6	81.0 81.9	59.9 61.3
1 TMS ether	80.8 81.2	76.0 76.0	72.2 73.0	71.7 72.0	81.4 82.6	61.7 62.8
2	77.3 79.3	75.1 75.7	72.0 72.9	70.6 71.1	82.3 82.3	62.1 62.2
2 TMS ether	80.8 81.2	75.9 76.0	72.2 73.0	71.7 71.9	81.4 82.5	61.7 62.8
3[b]	78.8	73.9	71.4	70.8	81.4	61.5
3 TMS ether	80.9	75.1	73.0	72.0	81.8	62.6
4[b]	79.0	73.4	70.7	70.7	81.3	61.6
5	79.1(101.5)	74.0(72.9)	71.2(77.4)	69.8(69.8)	81.1(76.1)	61.0(61.0)
5 TMS ether	81.5(100.0)	74.4(73.1)	72.0(78.9)	71.9(71.3)	82.7(77.3)	63.0(62.0)
6[b]	79.5	74.6	73.0	71.0	81.4	60.2
6 TMS ether	80.8	74.9	72.9	71.5	81.3	61.8
1 acetate	77.0 77.0	72.8 73.8	69.2 70.3	69.2 70.3	74.5 74.9	62.6 62.8

[a] The TMS ethers were measured in $CDCl_3$, the acetate in acetone-d_6 and the others in DMSO-d_6. [b] Measured in 1 mm capillary tube. [c,d] Assignments bearing the same superscript may be reversed, except for those in parentheses.

Table 3. ^{13}C NMR shifts of the sugar moieties of some glucosides.

Compound	C-1	C-2	C-3	C-4	C-5	C-6	Solvent
β-D-Glucopyranosylbenzene	81.6[b]	74.0	77.1	69.7	79.7[b]	60.9	D_2O[a]
β-D-Glucopyranosylbenzene TMS ether	83.0	76.9	79.6	71.7	81.4	62.6	$CDCl_3$
Tetraacetyl-β-D-glucopyranosylbenzene	80.0	72.5	74.0	68.4	75.9	62.1	$CDCl_3$
Phenyl-β-D-glucopyranoside	100.4	73.1	75.8[b]	69.6	76.3[b]	60.8	D_2O[a]

[a] Acetone-d_6 was used as the internal standard. [b] Assignments bearing the same superscript in any one spectrum may be reversed.

and M^+ at *m/e* 778 and at *m/e* 814 for *2* corresponding to the molecular weight of PM- and PDM-di-*C*-glucosyl derivatives of apigenin and luteolin, respectively. These MS data are in agreement with those reported for vicenin-2 (apigenin 6,8-di-*C*-β-D-glucopyranoside) and lucenin-2 (luteolin 6,8-di-*C*-β-D-glucopyranoside).[19]

EXPERIMENTAL

UV spectra were recorded on a Varian Cary 118 spectrophotometer and NMR spectra were measured with a Jeol FX-100 (^{13}C NMR) and a Jeol FX-60 (^1H NMR) pulsed FT spectrometer. The ^{13}C NMR spectra were determined at 25.05 MHz either in 5 mm tubes (12 – 205 mg in 0.5 ml) or in 1 mm capillary tubes (12 mg in 20 – 30 μl).* Chemical shifts were subsequently referred to external TMS on the basis of the chemical shifts of DMSO-d_6, $CDCl_3$ and acetone-d_6 relative to their reference (39.5, 77.0 and 29.8, respectively). Both proton noise-decoupled and off-resonance decoupled spectra have been recorded. Mass spectra were recorded as described earlier.[1] Isolation and separation of the flavonoids from the moss *H. ciliata* were described earlier.[1] Solvent system: BuOH – HOAc – H_2O, 6:1:2 (BAW), *t*-BuOH – HOAc – H_2O, 3:1:1 (TBA), CH_2Cl_2-acetone, 3:1 (MA).

Apigenin 6,8-di-C-β-D-glucopyranoside (vicenin-2, *1*). Gel filtration on Sephadex G-25 with EtOH-H_2O (1:1) as eluent gave 0.38 g of *1*, m.p. 214 – 216 °C (decomp.). $[\alpha]_D^{22}$ + 71° (*c* = 0.36, H_2O), $[\alpha]_D^{20}$ + 25° (*c* = 0.26, pyridine). *UV*

* All spectra were determined at 23 °C except for *3* (100 °C), *4* (50 °C) and *6* (80 °C).

(99.9 % MeOH): 272, 331; (+$AlCl_3$): 279, 304, 352, 385sh; (+$AlCl_3$/HCl): 279, 305, 346, 385sh; (+MeONa): 282, 333, 398; (+NaOAc): 282, 313sh, 335, 395; (+NaOAc/H_3BO_3): 275, 284sh, 321, 345sh, 406sh nm. ^{13}C *NMR* data, see Tables 1 and 2. R_F values: 0.15 (TBA), 0.22 (BAW) and 0.40 (15 % HOAc).

The acetate was prepared with Ac_2O in pyridine, m.p. 159–161 °C, $[\alpha]_D^{22}$ +16° (c = 1.22, $CHCl_3$). ^1H *NMR* (60 MHz, $CDCl_3$): δ 8.03 (H2′ and H6′, d, J = 8.5 Hz), 7.35 (H3′ and H5′, d, J = 8.5 Hz), 6.62 (H3, s), 6.2–3.7 (sugar H), 2.51, 2.45 and 2.31 (aromatic acetyl H), 2.06, 2.03, 2.01, 1.98, 1.95, 1.88, 1.84 and 1.72 (sugar acetyl H). ^{13}C *NMR* data, see Tables 1 and 2.

The TMS ether was prepared according to standard procedures.[2] ^1H *NMR* (60 MHz, $CDCl_3$): δ 7.89 (H2′ and H6′, d, J = 8.5 Hz), 6.95 (H3′ and H5′, d, J = 8.5 Hz), 6.46 (H3, s), 4.9–3.1 (sugar H). ^{13}C *NMR* data, see Tables 1 and 2.

The permethyl ether was prepared with NaH, DMSO and CH_3I,[20] and purified by TLC on silica gel in acetone. R_F value: 0.47 (MA). The mass spectra contained the following major ions, *m/e* (% rel. int.): 748 (3), 734 (8), 733 (19), 719 (9), 718 (32), 717 (85), 701 (8), 685 (6), 645 (20), 615 (9), 585 (60), 573 (100), 559 (16), 543 (18), 541 (20), 529 (5) and 527 (10).

The perdeuteriomethyl ether was prepared with NaH, DMSO and CD_3I,[20] and purified by TLC on silica gel in acetone. R_F value: 0.40 (MA). The mass spectra contained the following major ions, *m/e* (% rel. int.): 781 (7), 764 (14), 763 (32), 749 (13), 748 (40), 747 (100), 729 (9), 672 (18), 608 (32), 597 (95), 580 (12) and 561 (12).

Luteolin 6,8-di-C-β-D-glucopyranoside (lucenin-2, *2*). Gel filtration on Sephadex G-25 with EtOH-H_2O (1:1) as eluent gave 0.15 g of *2*, m.p. 265–267 °C (decomp.). $[\alpha]_D^{20}$ +16° (c = 0.062, pyridine). *UV* (99.9 % MeOH): 260sh, 271, 349; (+$AlCl_3$): 278, 305sh, 334sh, 429; (+$AlCl_3$/HCl): 279, 297sh, 363, 385sh; (+MeONa): 269, 280sh, 340sh, 410; (+NaOAc): 274sh, 281, 328sh, 403; (+NaOAc/H_3BO_3): 267, 287sh, 386, 427 nm. ^{13}C *NMR* data, see Tables 1 and 2. R_F values: 0.07 (TBA), 0.11 (BAW) and 0.25 (15 % HOAc).

The preparation of *the TMS ether*, *the permethyl ether* and *the perdeuteriomethyl ether* was analogous to that of the corresponding derivatives of *1*.

The TMS ether. ^1H *NMR* (60 MHz, $CDCl_3$): δ 7.76 (H6′, q, J = 8 Hz and 2 Hz), 7.20 (H2′, d, J = 2 Hz), 6.96 (H5′, d, J = 8 Hz), 6.40 (H3, s), 4.9–3.1 (sugar H). ^{13}C *NMR* data, see Tables 1 and 2.

The permethyl ether. R_F value: 0.42 (MA). The mass spectra contained the following major ions, *m/e* (% rel. int.): 778 (12), 764 (12), 763 (29), 749 (14), 748 (42), 747 (100), 731 (11), 715 (8), 675 (15), 595 (6), 615 (43), 603 (66), 589 (11), 573 (13), 571 (12), 559 (6) and 557 (9).

The perdeuteriomethyl ether. R_F value: 0.34 (MA). The mass spectra contained the following major ions, *m/e* (% rel. int.): 814 (6), 797 (13), 796 (26), 782 (14), 781 (42), 780 (85), 762 (8), 705 (16), 641 (41), 631 (40), 630 (100), 613 (6) and 594 (8).

Tetraacetyl-β-D-glucopyranosylbenzene was prepared as previously described.[21] M.p. 152–154 °C, $[\alpha]_D^{21}$ –13° (c = 1.21, $CHCl_3$), ^{13}C *NMR* (100 MHz, $CDCl_3$): 170.4, 170.0, 169.2, 168.5 (CO, acetyl), 136.1, 128.7, 128.2, 128.2, 126.9, 126.9 (benzene), 80.0, 75.9, 74.0, 72.5, 68.4, 62.1 (glucose), 20.4, 20.4, 20.4, 20.2 ppm (CH_3, acetyl).

β-D-Glucopyranosylbenzene was prepared from the tetraacetyl derivative.[22] ^{13}C *NMR* (100 MHz, D_2O): 137.6, 128.7, 128.6, 128.6, 127.8, 127.8 (benzene), 81.6, 79.7, 77.1, 74.0, 69.7, 60.9 ppm (glucose).

Saponarin (5) was isolated from the moss *Mnium undulatum*,[23] acid treatment of saponarin gave *isovitexin (4)* and *vitexin (3)*. *Apigenin (7)* was prepared by synthesis, and *orientin (6)* was a gift. Their TMS ethers were prepared according to standard procedures.[2]

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