Chemical Studies on Bryophytes. 21. Flavonoid Glycosides of *Hedwigia ciliata*

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Six flavonoid *O*-glycosides and one *C*-glycosides have been identified from the moss *Hedwigia ciliata*. Four of the *O*-glycosides are new natural products; one apigenin tetraglycoside and three triglycosides of apigenin and luteolin. The others are two earlier known apigenin and luteolin diglycosides. The *C*-glycoside is an apigenin 6,8-di-*C*-hexoside. The structures were determined using spectroscopic methods, sugar linkage analysis by GLC and hydrolytic experiments.

Twelve flavonoid compounds were isolated from the moss *Hedwigia ciliata*.[1] Earlier, the structures of two new luteolin tetraglycosides (*7* and *8*) [1,2] and two di-*C*-glucosides (*10* and

11) [3] were reported. The present communication treats the structure determinations of six flavonoid *O*-glycosides (*1 — 6*) and one *C*-glycoside (*9*) of the previously isolated flavonoids.

All the *O*-glycosides gave the same sugars, glucose and rhamnose, on hydrolysis. The aglycone isolated after hydrolysis was either apigenin (from *1 — 3*) or luteolin (from *4 — 6*).

UV spectral data of *1* indicate that the sugars are linked to apigenin at the 7-position, and this was confirmed by identification of 5,4'-di-*O*-methylapigenin after methylation and hydrolysis.[4] Partial hydrolysis of *1* gave one intermediate, *1a*, which was rapidly hydrolyzed by

Table 1. R_F values, acidic and enzymatic hydrolysis data of the isolated flavonoid O-glycosides and their partially hydrolyzed products.

Compound		Spot colour NH$_3$/UV	R_F values 15 % HOAc	TBA	Partial acidic hydrolysis products	Enzymatic hydrolysis products
1	Apigenin-7-*O*-neohesperidoside	Yellow	0.32	0.61	*1a* and apigenin	No hydrolysis
1a	Apigenin-7-*O*-β-D-glucoside	Yellow	0.11	0.46	Apigenin	Apigenin (fast)
2	Apigenin-7-*O*-neohesperidoside-4'-*O*-β-D-glucoside	Purple	0.63	0.18	*1, 1a, 2a* and apigenin	*1* (fast)
2a	Apigenin-7,4'-di-*O*-β-D-glucoside	Purple	0.34	0.15	*1a* and apigenin	Apigenin (fast)
3	Apigenin-7-*O*-neohesperidoside-4'-*O*-sophoroside	Purple	0.66	0.04	*1, 1a, 2, 2a, 3a* and apigenin	*1* (slowly)
3a	Apigenin-7-*O*-β-D-glucoside-4'-*O*-sophoroside	Purple	0.42	0.04		
4	Luteolin-7-*O*-neohesperidoside	Yellow	0.18	0.38	*4a* and luteolin	No hydrolysis
4a	Luteolin-7-*O*-β-D-glucoside	Yellow	0.06	0.26	Luteolin	Luteolin (fast)
5	Luteolin-7-*O*-neohesperidoside-4'-*O*-β-D-glucoside	Purple	0.48	0.15	*4, 4a* and luteolin	*4* (fast)
6	Luteolin-7-*O*-β-D-glucoside-4'-*O*-neohesperidoside	Purple	0.52	0.11	*6a, 4a* and luteolin	*6b* (fast)
6a	Luteolin-7,4'-di-*O*-β-D-glucoside	Purple	0.19	0.13	*4a* and luteolin	Luteolin (fast)
6b	Luteolin-4'-*O*-neohesperidoside	Purple	0.36	0.52		No hydrolysis

β-glucosidase to apigenin, indicating that *1a* is apigenin-7-*O*-β-D-glucopyranoside. This was confirmed by co-chromatography with an authentic sample. The mass spectra of permethylated *1* gave a molecular peak at m/e 690, and peaks for a rhamnoglucoside unit, m/e 393 and m/e 361.[5] To decide at which position the rhamnose unit was linked to the glucose, permethylated *1* was hydrolyzed and the methylated sugars were then reduced and acetylated. GLC analysis of the methylated alditolacetates showed that *1* has the rhamnose linked to the glucose at the 2-position.[6] Since it is most likely that the rhamnose is an α-L-rhamnose, the structure of *1* should be apigenin-7-*O*-neohesperidoside [apigenin-7-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside].

The other two apigenin-*O*-glycosides, *2* and *3*, have sugars linked in both the 7- and the 4′-positions according to UV spectral data and the identification of 5-*O*-methylapigenin after methylation and hydrolysis.[4] The ¹H NMR spectrum of the TMS ether of *3* has three β-glucose H-1 signals at δ 4.47, 5.13 and 5.31, and one α-rhamnose H-1 signal at δ 4.95. The doublet at δ 1.20 also indicates the presence of one rhamnose unit.[4] These data suggest that *3* is a tetraglycoside. That *2* is a triglycoside with two glucose units and one rhamnose unit was confirmed by the observation in the ¹³C NMR spectrum (Table 2) of the signals for C-6 in glucose and rhamnose (60.7, 60.4 and 18.4 ppm, respectively). The assignments of the sugar carbons in Table 2 are based on those given in the literature.[7-9]

Enzymatic hydrolysis of *2* with β-glucosidase gave *1* rapidly in contrast to *3* which was slowly hydrolyzed to *1*. The probable explanation is that *2* has one β-D-glucose unit and that *3* has a sophoroside [(2-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside] unit in the 4′-position.[10] Partial hydrolysis of *2* and *3* gave three and four partially hydrolyzed products, respectively, (Table 1) where one of the intermediates, *2a* with sugars in both the 7- and the 4′-positions, was rapidly hydrolyzed with β-glucosidase to apigenin. This shows that *2* and *3* have β-D-glucose units linked to the apigenin in both the 7- and the 4′-positions.

Mass spectroscopy of permethylated *2* and *3* gave only a molecular peak for the triglycoside *2* (m/e 894). The mass spectrum of permethylated *3* gave fragments at m/e 706 and m/e 676 from the aglycone with a diglucoside unit and a rhamnoglucoside unit, respectively. The corresponding peaks in the mass spectrum of *2* are at m/e 676 and m/e 502 for the aglycone with a rhamnoglucoside unit and a glucoside unit, respectively. In both spectra there are peaks from a rhamnoglucoside unit, m/e 393 and m/e 361. There are also fragments from a diglucoside unit in *3*, m/e 423 and m/e 391, while *2* only shows peaks from a terminal glucose unit, m/e 219, m/e 187 and m/e 155.[5]

GLC-MS analysis of the methylated alditolacetates gave the same results for *2* and *3*, except for peak areas. Both *2* and *3* gave methylated alditolacetates which correspond to terminal glucose, terminal rhamnose and 2-substituted glucose.[6] This analysis shows that

Table 2. ¹³C NMR shifts of the sugar moieties of some of the isolated flavonoid *O*-glycosides.[a]

Compound	C-1^G	C-2^G	C-3^G	C-4^G	C-5^G	C-6^G	C-1^R	C-2^R	C-3^R	C-4^R	C-5^R	C-6^R
2	100.7	80.0	76.7 ^{a}	69.9	77.5 ^{a}	60.7	97.8	69.9	70.6	73.2	68.5	18.4
	100.7	75.1	77.0 ^{a}	72.2	77.2 ^{a}	60.4						
4	102.2	76.4	77.2	70.6 ^{a}	77.2	60.5	98.3	70.2 ^{a}	70.6 ^{a}	73.2	68.9	18.2
5	100.7	79.7	76.0 ^{a}	70.4 ^{b}	77.1 ^{a}	60.7	98.1	70.0 ^{b}	70.6 ^{b}	73.1	68.2	17.7
	101.6	74.7	76.9 ^{a}	72.0	77.1 ^{a}	60.4						
7	100.6	76.5	77.2	70.6	77.2	60.7	98.1	69.8	70.6	72.2	68.7	18.2
	100.6	76.5	77.2	70.6	77.2	60.7	98.1	69.8	70.6	72.2	68.7	18.2
8	100.7	76.0	76.5 ^{a}	69.3 ^{b}	77.0 ^{a}	60.8	98.1	69.6 ^{b}	70.7	72.2	68.6	18.4
	100.7	82.0	76.5 ^{a}	69.3 ^{b}	77.3 ^{a}	60.8						
	104.1	74.7	77.0 ^{a}	70.7	77.3 ^{a}	60.8						

[a] G refers to glucose and R to rhamnose. Assignments bearing the same superscript in any spectrum may be reversed.

2 must be apigenin-7-*O*-neohesperidoside-4′-*O*-β-D-glucopyranoside and *3* apigenin-7-*O*-neohesperidoside-4′-*O*-sophoroside. Both *2* and *3* are new natural products.

As regards the luteolin derivatives (*4 − 6*), UV spectral data indicate that *4* has the sugars (glucose and rhamnose) linked only in the 7-position, while *5* and *6* have sugars in both the 7- and the 4′-positions.[4] This was also indicated by isolation of 5,3′,4′-tri-*O*-methylluteolin and 5,3′-di-*O*-methylluteolin, respectively, after methylation and hydrolysis. The only intermediate after partial hydrolysis of *4* was luteolin-7-*O*-β-D-glucopyranoside (*4a*), which was identified by hydrolysis with β-glucosidase to luteolin. Mass spectroscopy of permethylated *4* gave a molecular peak at m/e 720 and fragments at m/e 393 and m/s 361, indicating a luteolin-7-*O*-rhamnoglucoside. ^{13}C NMR spectroscopy of *4* (Table 2) also agreed with this conclusion. Cochromatography with an authentic sample established the structure of *4* as luteolin-7-*O*-neohesperidoside.

Compounds *5* and *6*, with the sugars linked both in the 7- and the 4′-positions, gave very similar mass spectra for the permethylethers. No molecular peaks could be seen, but there were peaks at m/e 706 and m/e 532 for luteolin linked to a rhamnoglucoside unit and to a glucoside unit, respectively. Other dominating peaks come from a rhamnoglucoside unit, m/e 393 and m/e 361, from a terminal rhamnose unit, m/e 189, m/e 157 and m/e 125, and from a terminal glucose unit, m/e 219, m/e 187 and m/e 155, as well as from the aglycone residue, m/e 314.[5] This showed that *5* and *6* must be triglycosides, which was confirmed for *5* by ^{13}C NMR spectroscopy (Table 2) and by ^{1}H NMR spectroscopy of the TMS ether.

Enzymatic hydrolysis of *5* with β-glucosidase gave in a short time luteolin-7-*O*-neohesperidoside (*4*) indicating the structure of *5* to be luteolin-7-*O*-neohesperidoside-4′-*O*-β-D-glucopyranoside, a new natural product.

Partial hydrolysis of *6* gave two intermediates *6a* and *4a*. UV spectral data of *6a* showed that it had both the 7- and the 4′-positions substituted;[4] enzymatic hydrolysis of *6a* with β-glucosidase rapidly gave luteolin. This showed that the structure of *6a* is luteolin-7,4′-di-*O*-β-D-glucopyranoside. Enzymatic hydrolysis of *6* with β-glucosidase gave a new intermediate *6b*,
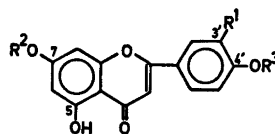
	R¹	R²	R³
1	H	Neohesperidosyl [a]	H
1a	H	β-D-Glucopyranosyl	H
2	H	Neohesperidosyl [a]	β-D-Glucopyranosyl
2a	H	β-D-Glucopyranosyl	β-D-Glucopyranosyl
3	H	Neohesperidosyl [a]	Sophorosyl [b]
3a	H	β-D-Glucopyranosyl	Sophorosyl [b]
4	OH	Neohesperidosyl [a]	H
4a	OH	β-D-Glucopyranosyl	H
5	OH	Neohesperidosyl [a]	β-D-Glucopyranosyl
6	OH	β-D-Glucopyranosyl	Neohesperidosyl [a]
6a	OH	β-D-Glucopyranosyl	β-D-Glucopyranosyl
6b	OH	H	Neohesperidosyl [a]
7	OH	Neohesperidosyl [a]	Neohesperidosyl [a]
8	OH	Neohesperidosyl [a]	Sophorosyl [b]

[a] (2-*O*-α-L-Rhamnopyranosyl)-β-D-glucopyranosyl. [b] (2-*O*-β-D-Glucopyranosyl)-β-D-glucopyranosyl.

with sugar units linked in the 4′-position, according to UV data.

GLC-MS analysis of the methylated alditolacetates derived from permethylated *6*, showed that *6* has terminal glucose and rhamnose units and a 2-substituted glucose unit.[6] The conclusion is that *6* must have a β-D-glucopyranoside linked in the 7-position and a neohesperidoside linked in the 4′-position. This establishes the structure of *6* as luteolin-7-*O*-β-D-glucopyranoside-4′-*O*-neohesperidoside, a new triglycoside of luteolin.

Treatment of *9* with diluted acid gave no aglycone or free sugar which suggests that *9* is a *C*-glycoside. Since no Wessely-Moser-rearrangement occurred during acid treatment, *9* has the same, or very similar, sugar units in the 6- and the 8-positions.[11] The aglycone moiety of *9* is apigenin according to UV data.[4] To obtain information on the sugar moieties, *9* was perdeuteriomethylated and submitted to mass spectroscopy analysis. The molecular peak at m/e 781 and the fragmentation data suggest that *9* is a 6,8-di-*C*-hexosylapigenin. The mass spectrum is identical to that of perdeuteriomethylated apigenin 6,8-di-*C*-β-D-glucopyranoside (*10*).[3] Since their R_F data are different, *9* cannot be identical with *10*. It is, however, difficult to distinguish between *C*-glucosylapigenin and *C*-

galactosylapigenin by the mass spectra of their permethyl ethers,[12] and probably also to distinguish between other C-hexosylapigenins. It is not possible with the amount of sample available to decide the structure of *9* closer than to an apigenin 6,8-di-C-hexoside.

EXPERIMENTAL

Isolation and *separation* of the flavonoids from the moss *H. ciliata* have been described earlier.[1] The crude flavone fractions were further purified by gelfiltration on Sephadex G-25 with EtOH−H$_2$O (1:1) as eluent. UV-visible and NMR spectra were recorded as previously described,[3] as well as the mass spectra and GLC.[1,2] Solvent system: t-BuOH−HOAc−H$_2$O, 3:1:1 (TBA). R_F values were determined on 0.1 mm pre-coated cellulose TLC plates (Merck) if not otherwise stated.

Acid hydrolysis was performed with 6 % HCl for 1 h at 100 °C and the products were identified by co-chromatography with authentic samples and the flavone was also determined by UV and mass spectra. *Enzymatic hydrolysis* was carried out at 37 °C in an acetate buffer solution (pH 5.0), using β-glucosidase (Koch-Light). *Partial acidic hydrolysis* was performed with 6 % HCl for 10−30 days at room temperature and the partially hydrolyzed products were then separated by TLC on cellulose in 15 % HOAc and TBA. Results from partial acidic hydrolysis and enzymatic hydrolysis of the partially hydrolyzed products are shown in Table 1.

The *TMS ethers* were prepared according to standard procedures,[4] and *the permethyl ethers* were prepared with NaH, DMSO and CH$_3$I according to Hakomori's procedure.[13] The permethyl ethers were purified by TLC on silica gel with acetone or CHCl$_3$−acetone (3:1) as eluent.

Linkage analysis of sugar. The permethylated glucosides were hydrolyzed with 8 % H$_2$SO$_4$ for 1 h at 100 °C. The aglycone was separated from the methylated sugars by polyamide column chromatography as described earlier[2] and identified by mass spectra, UV and R_F values. The methylated sugars were reduced with NaBH$_4$ and acetylated with Ac$_2$O in

pyridine to methylated alditolacetates as described earlier.[6] The alditolacetates were identified by GLC or GLC-MS analysis. T-values are relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol ($T=1.00$).[6]

Apigenin-7-O-neohesperidoside (1). 20 mg, R_F values, see Table 1. UV (99.9 % MeOH): 268, 332; (+AlCl$_3$): 275, 299, 348, 381; (+AlCl$_3$/HCl): 276, 298, 343, 380; (+MeONa): 255sh, 267, 387; (+NaOAc): 267, 387; (+NaOAc/H$_3$BO$_3$): 267, 338 nm. *Acid hydrolysis* of *1* gave apigenin, glucose and rhamnose. *Partial acidic hydrolysis* of *1* gave, besides *1* and apigenin, one intermediate *1a*. *1 permethyl ether*. MS [14 eV, 140 °C; m/e (% rel. int.)]: 692 (3), 691 (6), 690 (14), 659 (2), 488 (4), 487 (14), 395 (3), 394 (12), 393 (61), 363 (4), 362 (18), 361 (100), 333 (3), 332 (4), 300 (2), 299 (10), 298 (12), 297 (2), 190 (8), 189 (73), 188 (5), 158 (2), 157 (21), 145 (2). Only peaks larger than 2 % of the base peak are given. *Linkage analysis of sugar.* GLC analysis of the methylated alditolacetates gave 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol ($T=0.48$) and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl-D-glucitol ($T=1.95$). The aglycone was identified as 5,4'-di-O-methyl-apigenin.

Apigenin-7-O-neohesperidoside-4'-O-β-D-glucopyranoside (2). 53 mg, R_F values, see Table 1. UV (99.9 % MeOH): 270, 316; (+AlCl$_3$): 277, 297, 339, 376sh; (+AlCl$_3$/HCl): 278, 297, 335, 375sh; (+MeONa): 288, 379; (+NaOAc): 270, 311, 396sh; (+NaOAc/H$_3$BO$_3$): 269, 320, 398sh nm. ^{13}C NMR (100 MHz, DMSO-d_6): Sugar C, see Table 2. *Acid hydrolysis* of *2* gave apigenin, glucose and rhamnose. *Enzymatic hydrolysis* of *2* gave *1* rapidly ($<1\frac{1}{2}$ h). *Partial acidic hydrolysis* of *2* gave, besides *2* and apigenin, three intermediates *1*, *1a* and *2a*, where *1* was the main component. *2 permethyl ether*. MS [16 eV, 130 °C; m/e (% rel. int.)]: 894 (1), 690 (1), 676 (2), 657 (4), 626 (4), 625 (4), 502 (4), 393 (15), 362 (6), 361 (22), 360 (6), 284 (10), 219 (16), 218 (27), 204 (10), 190 (12), 189 (100), 188 (42), 187 (62), 157 (28), 155 (9), 145 (10), 129 (7), 127 (6), 125 (6). Only peaks larger than 6 % (1 % for m/e 500−1000) of the base peak are given. *Linkage analysis of sugar.* GLC analysis of the methylated alditolacetates gave 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol ($T=0.46$), 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol ($T=1.01$) and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl-D-glucitol ($T=1.92$). The aglycone was identified as 5-O-methylapigenin.

Apigenin-7-O-neohesperidoside-4'-O-sophoroside (3). 58 mg, $[\alpha]_D^{26}$ −63.2° (c 1.24, H$_2$O), R_F values, see Table 1. UV (99.9 % MeOH): 269, 322; (+AlCl$_3$): 277, 296, 341, 379sh; (+AlCl$_3$/HCl): 279, 295, 336, 379sh; (+MeONa): 282, 365 (+NaOAc): 269, 318; (+NaOAc/H$_3$BO$_3$): 269, 323 nm. *Acid hydrolysis* of *3* gave apigenin, glucose and rhamnose. *Enzymatic hydrolysis* of *3* gave *1* slowly; only slight hydrolysis was observed after 24 h and it was
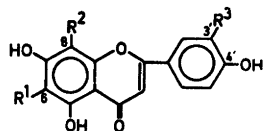
	R^1	R^2	R^3
9	Hexosyl	Hexosyl	H
10	β-D-Glucopyranosyl	β-D-Glucopyranosyl	H
11	β-D-Glucopyranosyl	β-D-Glucopyranosyl	OH

not hydrolyzed completely even after 3 weeks. *Partial acidic hydrolysis* of *3* gave, besides *3* and apigenin, five intermediates, *1, 1a, 2, 2a* and *3a*, where *1* was the main component and *2a* and *3a* were only obtained in very small quantities. *3 TMS ether.* ^1H *NMR* (100 MHz, $CDCl_3$): δ 7.75 (H2', H6', J 4.5 Hz), 7.09 (H3', H5', J 4.5 Hz), 6.78 (H8, J 1 Hz), 6.48 (H3), 6.41 (H6, J 1 Hz), 5.31 (glucose H1, J 6 Hz), 5.13 (glucose H1, J 6 Hz), 4.95 (rhamnose H1, J 1 Hz), 4.47 (glucose H1, J 7 Hz), 4.0 − 2.8 (sugar H). 1.20 (rhamnose CH_3, J 5 Hz). *3 permethyl ether.* MS [70 eV, 175 °C; m/e (% rel. int.)]: 706 (5), 676 (5), 423 (1), 393 (5), 392 (5), 391 (21), 362 (3), 361 (14), 328 (4), 315 (3), 314 (7), 298 (5), 285 (4), 284 (8), 219 (6), 218 (7), 207 (4), 204 (3), 201 (3), 189 (68), 188 (13), 187 (69), 157 (35), 155 (19), 145 (19), 131 (11), 129 (14), 127 (16), 125 (13), 115 (17), 113 (13), 111 (46), 102 (12), 101 (100). Only peaks larger than 10 % (3 % for m/e 200 − 1200) of the base peak are given. *Linkage analysis of sugar.* GLC-MS analysis of the methylated alditolacetates gave 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol (T = 0.47), 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol (T = 1.02) and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl-D-glucitol (T = 1.97). The aglycone was identified as 5-O-methylapigenin.

Luteolin-7-O-neohesperidoside (4). 31 mg, R_F values, see Table 1. UV (99.9 % MeOH): 255, 265sh, 349; (+AlCl₃): 272, 296sh, 331sh, 427; (+AlCl₃/HCl): 265sh, 272, 295sh, 363, 385; (+MeONa): 267, 404; (+NaOAc): 259, 404; (+NaOAc/H₃BO₃): 257, 372 nm. ^{13}C *NMR* (100 MHz, DMSO-d_6): sugar C, see Table 2. *Acid hydrolysis* of *4* gave luteolin, glucose and rhamnose. *Partial acidic hydrolysis* of *4* gave, besides *4* and luteolin, only one intermediate, *4a*. *4 permethyl ether.* [MS 70 eV, 130 °C; m/e (% rel. int.)]: 720 (1), 689 (1), 531 (1), 393 (7), 368 (5), 362 (7), 361 (30), 330 (12), 329 (29), 328 (62), 327 (31), 315 (21), 314 (31), 311 (16), 301 (24), 299 (28), 283 (17), 282 (23), 237 (16), 223 (20), 201 (15), 191 (20), 190 (16), 189 (100), 188 (17), 187 (25), 185 (15), 175 (15), 167 (20), 165 (21), 163 (18), 161 (17), 159 (18), 157 (63), 155 (24), 151 (16), 149 (64), 147 (26), 145 (45), 143 (21), 141 (26). Only peaks larger than 15 % (5 % for m/e 350 − 450 and 1 % for m/e 450 − 800) of the base peak are given. *Linkage analysis of sugar.* GLC analysis of the methylated alditolacetates gave 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol (T = 0.46) and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl-D-glucitol (T = 1.92). The aglycone was identified as 5,3',4'-tri-O-methylluteolin.

Luteolin-7-O-neohesperidoside-4'-O-β-D-glucopyranoside (5). 113 mg, $[\alpha]_D^{24}$ −66.0° (c 0.15, pyridine), R_F values, see Table 1. UV (99.9 % MeOH): 239sh, 249sh, 269, 334; (+AlCl₃): 261sh, 278, 293sh, 351, 380sh; (+AlCl₃/HCl): 262sh, 276, 293sh, 346, 380sh; (+MeONa): 266, 318, 371; (+NaOAc): 266, 332; (+NaOAc/H₃BO₃): 268, 336 nm. ^{13}C *NMR* (100 MHz,

DMSO-d_6): 181.8 (C-4), 163.8 (C-2), 163.0 (C-7), 161.0 (C-5), 157.1 (C-8a), 148.7 (C-4'), 147.3 (C-3'), 124.9 (C-1'), 118.4 (C-6'), 116.7 (C-5'), 113.9 (C-2'), 105.7 (C-4a), 104.4 (C-3), 100.4 (C-6), 94.2 (C-8), sugar C, see Table 2. *Acid hydrolysis* of *5* gave luteolin, glucose and rhamnose. *Enzymatic hydrolysis* of *5* gave *4* rapidly (< 1 h). *Partial acidic hydrolysis* of *5* gave, besides *5* and luteolin, two intermediates *4* and *4a*. *5 TMS ether.* ^1H *NMR* (100 MHz, CDCl₃): δ 7.40 (H6'), 7.25 (H2'), 6.95 (H5'), 6.72 (H8), 6.42 (H3), 6.38 (H6), 5.18 (glucose H1, J 6 Hz), 5.00 (glucose H1, J 6 Hz), 4.92 (rhamnose H1, J 2 Hz), 4.4 − 3.1 (sugar H), 1.22 (rhamnose CH_3, J 5 Hz). *5 permethyl ether.* MS [70 eV, 140 °C; m/e (% rel. int.)]: 707 (8), 706 (12), 533 (2), 532 (4), 395 (1), 394 (2), 393 (8), 362 (4), 361 (23), 329 (6), 328 (16), 315 (13), 314 (52), 219 (5), 218 (24), 201 (7), 190 (10), 189 (100), 188 (12), 187 (28), 173 (5), 157 (41), 155 (12), 145 (20), 143 (6), 141 (7), 131 (10), 129 (16), 127 (11), 125 (14). Only peaks larger than 5 % (1 % for m/e 350 − 800) of the base peak are given. *Linkage analysis of sugar.* GLC-MS analysis of the methylated alditolacetates gave 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol (T = 0.44), 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol (T = 0.97) and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl-D-glucitol (T = 1.87). The aglycone was identified as 5,3'-di-O-methylluteolin.

Luteolin-7-O-β-D-glucopyranoside-4'-O-neohesperidoside (6). 19 mg, R_F values, see Table 1. UV (99.9 % MeOH): 250sh, 269, 334; (+AlCl₃): 260sh, 276, 293sh, 350, 384sh; (+AlCl₃/HCl): 260sh, 277, 293sh, 346, 383sh; (+MeONa): 267, 317sh, 375; (+NaOAc): 267, 328; (+NaOAc/H₃BO₃): 268, 337 nm. *Acid hydrolysis* of *6* gave luteolin, glucose and rhamnose, *Enzymatic hydrolysis* of *6* gave *6b* rapidly (< 1 h). *Partial acidic hydrolysis* of *6* gave, besides *6* and luteolin, *6a* and *4a*. *6 permethyl ether.* MS [14 eV, 155 °C; m/e (% rel. int.)]: 706, (5), 546 (9), 533 (18), 532 (47), 518 (5), 393 (17), 362 (9), 361 (29), 360 (9), 329 (11), 328 (28), 315 (14), 314 (31), 285 (8), 283 (13), 219 (19), 218 (55), 204 (10), 190 (9), 189 (100), 188 (28), 187 (35), 158 (9), 157 (29), 155 (10). Only peaks larger than 8 % (5 % for m/e 350 − 800) of the base peak are given. *Linkage analysis of sugar.* GLC analysis of the methylated alditolacetates gave 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol (T = 0.46), 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol (T = 1.01) and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl-D-glucitol (T = 1.94). The aglycone was identified as 5,3'-di-O-methylluteolin.

Luteolin-7,4'-di-O-β-D-glucopyranoside (6a). UV (99.9 % MeOH): 269, 331; (+MeONa): 271, 358 nm.

Luteolin-4'-O-neohesperidoside (6b). UV (99.9 % MeOH): 269, 331; (+MeONa): 270, 347sh, 368 nm.

Luteolin-7,4'-di-O-neohesperidoside (7). ^{13}C NMR (100 MHz, DMSO-d_6): 181.8 (C-4), 163.9

(C-2), 162.7 (C-7), 161.9 (C-5), 157.2 (C-8a), 148.7 (C-4'), 147.9 (C-3'), 124.2 (C-1'), 118.2 (C-6'), 116.1 (C-5'), 113.9 (C-2'), 105.9 (C-4a), 104.3 (C-3), 99.8 (C-6), 94.3 (C-8), sugar C, see Table 2.

Luteolin-7-O-neohesperidoside-4'-O-sophoroside (8). ^{13}C *NMR* (100 MHz, DMSO-d_6): 182.2 (C-4), 163.9 (C-2), 162.8 (C-7), 161.3 (C-5), 157.3 (C-8a), 148.7 (C-4'), 147.3 (C-3'), 125.0 (C-1'), 119.0 (C-6'), 116.4 (C-5'), 113.6 (C-2'), 105.8 (C-4a), 104.8 (C-3), 99.9 (C-6), 94.9 (C-8), sugar C, see Table 2.

Apigenin 6,8-di-C-hexoside (9). <1 mg, R_F values: 0.34 (15 % HOAc), 0.13 (TBA). *UV* (99.9 % MeOH): 272, 331; (+AlCl₃): 279, 305sh, 351, 385sh; (+AlCl₃/HCl): 279, 304sh, 347, 385sh; (+MeONa): 282, 333, 397; (+NaOAc): 281, 335sh, 393; (+NaOAc/H₃BO₃): 274, 283sh, 321, 350sh nm. No hydrolysis or isomerisation was observed with treatment with 6 % HCl at 100 °C for 5 h. *9 perdeuteriomethyl ether. R_F* values on silica gel in acetone: 0.59. *MS* [70 eV, 110 °C; *m/e* (% rel. int.)]: 782 (8), 781 (20), 780 (6), 764 (11), 763 (26), 762 (7), 749 (10), 748 (35), 747 (90), 746 (19), 733 (4), 730 (5), 729 (11), 715 (4), 714 (6), 713 (4), 712 (6), 694 (4), 677 (3), 673 (6), 672 (18), 671 (5), 640 (4), 639 (6), 620 (4), 619 (4), 612 (6), 611 (13), 610 (7), 609 (17), 608 (47), 607 (9), 599 (10), 598 (42), 597 (100), 596 (22), 593 (5), 592 (11), 591 (3), 581 (6), 580 (15), 579 (6), 578 (7), 577 (8), 576 (10), 565 (4), 564 (9), 563 (5), 562 (9), 561 (15). Only peaks larger than 3 % of the base peak are given.

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