Acta Chemica Scandinavica B 31 (1977) 293—296

Chemical Studies on Bryophytes. 18. Luteolin 7-*O*-Neohesperidoside-4′-*O*-sophoroside, another New Tetraglycoside from *Hedwigia ciliata*

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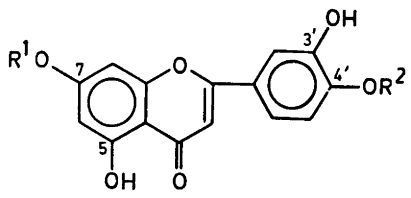
A new luteolin tetraglycoside from the moss *H. ciliata* has been identified. The structure is luteolin 7-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside-4′-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside (*1*) as determined using spectroscopic methods, sugar sequence analysis by GLC-MS and hydrolytic experiments.

In a previous contribution,[1] the isolation and separation of twelve flavonoid compounds from the moss *H. ciliata* and the structural determination of one of these, a luteolin 7,4′-di-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (*2*), has been described. This paper reports the structure of another new luteolin tetraglycoside from the moss *H. ciliata*.

Acidic hydrolysis of *1* gave luteolin, glucose and rhamnose. UV spectral studies with diagnostic shift reagents show that the sugars are linked to the aglycone at the 7- and 4′-positions.[2] This was confirmed by permethylation followed by hydrolysis, whereby 5,3′-di-*O*-methylluteolin was obtained. The high R_F value in 15 % HOAc, and the elemental analysis indicated that *1* is probably a luteolin tetraglycoside.

Mass spectroscopy of permethylated *1* gave peaks indicating that *1* has both a rhamnoglucoside unit, *m/e* 393 and *m/e* 361, and a diglucoside unit, *m/e* 423 and *m/e* 391.[3] There are also peaks from a terminal glucose unit, *m/e* 219, *m/e* 187 and *m/e* 155, and peaks from a terminal rhamnose unit, *m/e* 189, *m/e* 157 and *m/e* 125.



	R^1	R^2
1	neohesperidosyl [a]	sophorosyl [b]
1a	neohesperidosyl [a]	β-D-glucosyl
1b	β-D-glucosyl	sophorosyl [b]
1c	neohesperidosyl [a]	H
1d	β-D-glucosyl	H
1e	H	sophorosyl [b]
2	neohesperidosyl [a]	neohesperidosyl [a]

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

The ^{1}H NMR spectrum of the TMS ether of *1* showed that it is a luteolin tetraglycoside on the basis of the four sugar C-1 proton doublets at δ 5.52, 5.15, 4.86 and 4.55. The doublet at δ 1.21 and the signal at δ 4.86 ($J = 2$ Hz), indicate the presence of one α-rhamnose unit in 1.[2] The other three C-1 proton doublets ($J = 6$ Hz) must derive from glucose, since this coupling constant is characteristic for the C-1 proton of β-linked glucose.[4] Furthermore, the values of δ 5.52 and 5.15 for two of the C-1 protons indicate that two β-glucose units are linked to luteolin.

To establish the position of the interglycosidic linkages, a method described earlier [1,7–9] was used. After permethylation and hydrolysis,

Table 1. GLC-MS analysis data of the methylated alditol acetates. *T* values are relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

Alditol acetate	*T*	*T* [9]	Ratio of peak areas	Prominent fragments, *m/e*
1,5-Di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-rhamnitol	0.47	0.46	1.2	175, 161, 131, 117, 115, 101, 89, 43
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol	0.99	1.00	1.0	205, 161, 145, 129, 117, 101, 99, 87, 71, 45, 43
1,2,5-Tri-*O*-acetyl-3,4,6-tri-*O*-methyl-D-glucitol	1.90	1.98	2.0	205, 189, 161, 145, 129, 101, 99, 87, 45, 43

the methylated sugars were separated from the aglycon by polyamide column chromatography, and then reduced and acetylated. GLC-MS analysis gave three different alditol acetates in the peak area ratio of 1:1:2 (Table 1). This analysis indicates that *1* has one rhamnose and one glucose as terminal sugar units and two glucose units substituted in the 2-position.

Enzymatic hydrolysis of *1* with β-glucosidase was very slow and gave luteolin 7-*O*-neohesperidoside [luteolin-7-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside] (*1c*), identified by UV spectral studies, sugar determination and partial hydrolysis. The slow enzymatic hydrolysis of *1* indicated a sophoroside [(2-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside] unit in the 4′-position.[5] These results suggest that *1* is a luteolin-7-*O*-neohesperidoside-4′-*O*-sophoroside. This was confirmed by identification of sophorose and neohesperidose after H_2O_2 oxidation of *1*,[6] and by identification of the products obtained on acid hydrolysis (Table 2). Enzymatic hydrolysis of the partially hydrolyzed products show that the three glucose units in *1* are β-D-glucose.

The optical rotation value, $[\alpha]_D^{25}$ −90.1° (*c* 1.34, H_2O), of *1* compared with the isolated luteolin 7,4′-di-*O*-neohesperidoside (*2*),[1] $[\alpha]_D^{25}$ −86.5° (*c* 1.59, H_2O), indicated that the rhamnose unit in *1* must be α-L-rhamnose, since the contribution of the rotation from an α-L-rhamnose is of nearly the same magnitude and in the same direction as with a β-D-glucose.[10]

Considering these data, the structure of *1* is proposed to be luteolin 7-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glycopyranoside-4′-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside.

EXPERIMENTAL

NMR, UV and mass spectra were recorded as described earlier.[1] Isolation and separation of the flavonoids from the moss *H. ciliata* were described in the previous paper of this series.[1] Solvent systems: BuOH − HOAc − H_2O, 6:1:2 (BAW), *t*-BuOH − HOAc − H_2O, 3:1:1 (TBA), BuOH − HOAc − H_2O, 4:1:5 top layer (BAW/4:1:5).

Luteolin 7-O-neohesperidoside-4′-O-sophoroside (1). Gel filtration on Sephadex G-25 with EtOH − H_2O (1:1) as eluent and recrystallization from EtOH − H_2O (1:1) gave 0.56 g of *1*. Drying at 110 °C/0.1 mmHg over P_2O_5 gave a very hygroscopic compound, m.p. 219 − 223 °C, $[\alpha]_D^{25}$ −90.1° (*c* 1.34, H_2O). Found: C 50.68; H 5.75. Calc. for $C_{39}H_{60}O_{25}$: C 50.98; H 5.49. UV (99.9 % MeOH): 269, 334; (+ $AlCl_3$): 277, 293 sh, 349, 379 sh; (+ $AlCl_3$ − HCl): 278, 292 sh, 344, 379 sh; (+ MeONa): 268, 375; (+ NaOAc): 266, 328; (+ NaOAc − H_3BO_3): 266, 330 nm. R_F values, see Table 2.

Acidic hydrolysis of *1* with 6 % HCl at 100 °C for 1 h gave luteolin, glucose and rhamnose. These were identified by co-chromatography with authentic samples.

Partial hydrolysis of *1* with 6 % HCl at room temperature for 10 days gave, besides luteolin and *1*, four intermediates; *1a*, *1b*, *1c*, and *1d*, where *1c* was obtained in a larger quantity than the others. The partially hydrolyzed products were separated by PC in 15 % HOAc and gel filtrated. R_F values, spot colour in presence of NH_3 in UV light, and results from partial acid hydrolysis of the intermediates are shown in Table 2.

Enzymatic hydrolysis was carried out at 37 °C in an acetate buffer solution (pH 5.0). *1* was slowly hydrolyzed to *1c*; only slight hydrolysis was observed after 24 h and it was not hydrolyzed completely even after 20 days; *1a* was rapidly hydrolyzed (< 2 h) to *1c*; *1b* was rapidly hydrolyzed (< 2 h) to *1e*, which was slowly hydrolyzed to luteolin. Finally, *1d* was rapidly hydrolyzed (< 2 h) to luteolin.

Table 2. R_F values and acidic hydrolysis data of *1* and its partially hydrolyzed products.

Compound		Spot colour NH_3/UV	R_F values [a] 15 % HOAc	BAW	TBA	Partial acidic hydrolysis products
1	Luteolin 7-*O*-neohesperidoside-4′-*O*-sophoroside	purple	0.63	0.14	0.08	*1a*, *1b*, *1c*, *1d* and luteolin
1a	Luteolin 7-*O*-neohesperidoside-4′-*O*-glucoside	purple	0.49	0.27	0.20	*1c*, *1d* and luteolin
1b	Luteolin 7-*O*-glucoside-4′-*O*-sophoroside	purple	0.33	0.13	0.07	*1d* and luteolin
1c	Luteolin 7-*O*-neohesperidoside	yellow	0.19	0.42	0.41	*1d* and luteolin
1d	Luteolin 7-*O*-glucoside	yellow	0.06	0.37	0.29	luteolin
1e	Luteolin 4′-*O*-sophoroside	purple	0.17	0.40	0.34	luteolin

[a] R_F values on 0.1 mm pre-coated cellulose TLC plates.

UV spectral data. 1a (99.9 % MeOH): 266, 270, 334; (+ $AlCl_3$): 266, 272, 293 sh, 349, 383 sh; (+ $AlCl_3$ – HCl): 266, 272, 293 sh, 345, 383 sh; (+ MeONa): 266, 269, 375; (+ NaOAc): 266, 269, 333; (+ NaOAc – H_3BO_3): 266, 269, 334 nm. *1b* (99.9 % MeOH): 266, 269, 334; (+ $AlCl_3$): 266, 276, 294 sh, 349, 381 sh; (+ $AlCl_3$ – HCl): 267, 276, 294 sh, 344, 381 sh; (+ MeONa): 267 sh, 272, 378; (+ NaOAc): 267, 270, 333; (+ NaOAc – H_3BO_3): 267, 270, 333 nm. *1c* (99.9 % MeOH): 253, 267, 348; (+ $AlCl_3$): 274, 295 sh, 332, 431; (+ $AlCl_3$ – HCl): 274, 295 sh, 361, 388; (+ MeONa): 268, 405; (+ NaOAc): 259, 360 sh, 405; (+ NaOAc – H_3BO_3): 259, 371 nm. *1d* (99.9 % MeOH): 252, 266, 269 sh, 347; (+ $AlCl_3$): 267 sh, 273, 295 sh, 333, 427; (+ $AlCl_3$ – HCl): 266, 273, 295 sh, 360, 385; (+ MeONa): 266, 269 sh, 403; (+ NaOAc): 257, 265, 269 sh, 403; (+ NaOAc – H_3BO_3): 257, 265 sh, 269 sh, 370 nm. *1e* (99.9 % MeOH): 269, 331; (+ $AlCl_3$): 277, 293 sh, 348, 385 sh; (+ $AlCl_3$ – HCl): 275, 293 sh, 344, 385 sh; (+ MeONa): 267, 357; (+ NaOAc): 274, 317 sh, 351; (+ NaOAc – H_3BO_3): 269, 332 nm.

H_2O_2 oxidation. Two mg of *1* was dissolved in 0.5 ml of 0.1 M NH_3 and two drops of H_2O_2 (35 %) was added. After 4 h at room temperature the solution was chromatographed on Whatman No. 1 paper in BAW/4:1:5 for 48 h with glucose as reference.[6] H_2O_2 oxidation of *1* gave sophorose, R_g value 0.59 (lit.[6] 0.58), and neohesperidose, R_g value: 0.83 (lit.[6] 0.87). On comparison *2* gave only neohesperidose with R_g value 0.84, as expected.

The TMS ether was prepared according to standard procedures.[2] ^1H NMR (100 MHz, CCl_4): δ 7.38 (H6′), 7.27 (H2′), 7.14 (H5′), 6.68 (H8), 6.33 (H6), 6.29 (H3), 5.52 (glucose H1, *J* 6 Hz), 5.15 (glucose H1, *J* 6 Hz), 4.86 (rhamnose H1, *J* 2 Hz), 4.55 (glucose H1, *J* 6 Hz), 4.10 – 2.90 (22 sugar H), 1.21 (rhamnose CH_3, *J* 6 Hz).

The permethyl ether was prepared with NaH, DMSO and CH_3I according to Hakomori's procedure.[6] The permethyl ether was purified by TLC on silica gel with acetone as eluent. MS [15 eV; *m/e* (% rel. int.)]: 423 (3), 399 (3), 394 (4), 393 (19), 392 (7), 391 (36), 390 (3), 385 (3), 370 (5), 362 (10), 361 (35), 360 (3), 329 (3), 328 (10), 315 (9), 314 (34), 313 (6), 300 (6), 219 (11), 218 (29), 209 (12), 204 (11), 195 (12), 190 (11), 189 (100), 188 (27), 187 (100), 181 (15), 157 (31), 155 (15), 145 (11), 131 (6), 129 (7), 127 (10), 125 (6), 116 (15), 111 (22), 101 (35), 99 (26), 89 (9), 88 (12), 87 (9), 75 (19), 74 (11), 73 (6), 72 (7), 71 (15), 59 (9). Only peaks larger than 6 % (3 % *m/e* 300 – 510) of the base peak are given.

Linkage analysis of sugar. The permethylated glycoside was hydrolyzed with 8 % H_2SO_4 at 100 °C for 1 h. The reaction mixture was then passed through a polyamide column (Woelm, 0.5 × 2 cm), packed in water. The acidic sugar solution was eluted with water until the eluate was neutral, the aglycone being eluted with CH_3OH. After neutralisation with $BaCO_3$, the aqueous sugar solution was reduced with $NaBH_4$ and acetylated with Ac_2O in pyridine as described earlier.[6] Retention times and prominent fragments in GLC-MS are given in Table 1. The isolated aglycone was identified as 5,3′-di-*O*-methylluteolin by R_F values and UV spectral data.

Acknowledgements. We wish to thank Professor Gerd Bendz and Doc. Erling Nilsson for stimulating discussions. Elemental analyses were performed by the Analytical Department of the Chemical Institute, Uppsala, and the 100 MHz NMR spectrum is recorded at the Royal Agricultural College, Uppsala. Support from the Swedish Natural Science Research Council (G. Bendz) is gratefully acknowledged.

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Received November 10, 1976.