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Chemical Studies on Bryophytes. 20. A New Branched Flavonoid-*O*-triglycoside from *Dicranum scoparium*

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A new diosmetin (4′-*O*-methylluteolin) triglycoside has been isolated from the moss *Dicranum scoparium*. Using spectroscopic methods, sugar linkage analysis by GLC and hydrolytic experiments the flavonoid was identified as diosmetin 7-*O*-[2,4-di-*O*-(α-L-rhamnopyranosyl)]-β-D-glucopyranoside (*1*).

Three flavonoids were earlier isolated from the moss *D. scoparium*.[1,2] This paper reports the structure of a new flavone triglycoside (*1*) from *D. scoparium*. UV spectral studies of *1* with diagnostic shift reagents indicated a flavonoid substituted in the 7- and 4′-positions.[3] Acidic hydrolysis of *1* gave glucose, rhamnose and diosmetin (4′-*O*-methylluteolin). Partial hydrolysis of *1* gave two intermediates, *1a* and *1b*, the latter was hydrolyzed with β-glucosidase to diosmetin showing that *1* has a β-D-glucose unit linked in the 7-position.

The ^{13}C NMR spectrum of *1* in DMSO-d_6 confirmed that it was a glycoside of diosmetin. The ^{13}C NMR shifts of the aglycone part of *1* correspond well to the shifts of diosmetin,[4] the only significant differences being an upfield shift of 2.0 ppm for the C-7 signal and a downfield shift of 1.8 ppm for the *para*-related C-4a. These shifts are analogous to those reported when the 7-hydroxyl group is glycosylated in flavonoids.[5,6] The ^{13}C NMR spectrum also shows that there are one glucose and two rhamnose units in *1* on the basis of the signals for C-6 in glucose and rhamnose (59.7, 18.2 and 17.9 ppm respectively). The assignments of the sugar carbon in Table 1, are based on those given in the literature.[5–7] For comparison, the ^{13}C NMR spectrum of the earlier isolated apigenin 7-*O*-[2,4-di-*O*-(α-L-rhamnopyranosyl)]-β-D-glucopyranoside (*2*) was recorded. The sugar moieties of the two triglycosides show a very close relationship to each other, indicating that *1* has the same linkages between the sugars as *2*.

The mass spectra of permethylated *1* gave no molecular peak but fragments at *m/e* 567, 535, 503 and *m/e* 328, 329 for the sugar residue and the aglycone residue, respectively.[8] Only peaks from a terminal rhamnose unit, *m/e* 189,

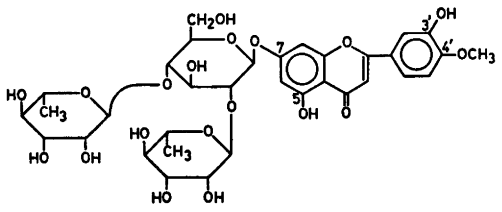
Table 1. ^{13}C NMR shifts of the sugar moieties [a] of the isolated flavonoids.

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
1	100.8 [b]	75.6	75.6	70.5	77.1	59.7	100.4 [b]	70.5	70.5	71.9	68.7	18.2
							97.4	70.5	70.5	71.9	68.4	17.9
2	100.8 [b]	75.7	75.7	70.7 [c]	77.1	59.7	100.5 [b]	70.5 [c]	70.7 [c]	72.0	68.5	18.2
							97.6	70.5 [c]	70.7 [c]	72.0	68.5	17.9

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

157 and 125, can be seen. There are also peaks at *m/e* 516 and 517 for the aglycone linked with a disubstituted glucose unit, indicating a branched sugar residue. The mass spectra of permethylated *2* are very similar to that of permethylated *1*.

To establish the position of the interglucosidic linkages, permethylated *1* was hydrolyzed and the methylated sugars were then reduced and acetylated. GLC analysis of the methylated alditol acetates gave 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-rhamnitol and 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl-D-glucitol.[9] This analysis shows that *1* has a branched sugar residue with two rhamnose units linked to glucose at the 2- and 4-positions, which is the same as in *2*.



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Considering these data, the structure of *1* is proposed to be diosmetin 7-*O*-[2,4-di-*O*-(α-L-rhamnopyranosyl)]-β-D-glucopyranoside.

EXPERIMENTAL

UV-visible spectra were recorded on a Varian Cary 118 spectrophotometer and ^{13}C NMR spectra were measured with a Jeol FX-100 FT spectrometer at 25.05 MHz in 5 mm tubes. Chemical shifts were referred to external TMS on the basis of the chemical shifts of DMSO-d_6 (39.5 ppm). Mass spectra and GLC were recorded as described earlier.[1] Solvent system: *t*-BuOH – HOAc – H_2O, 3:1:1 (TBA). R_F values were determined on 0.1 mm pre-coated cellulose TLC plates (Merck).

Isolation and *separation* of the flavonoids from the moss *D. scoparium* have been described earlier.[1] Approximately 25 mg of a flavone glycoside (*1*) was isolated from the crude fraction of luteolin 7-*O*-rhamnoglucoside. In contrast to the luteolin 7-*O*-rhamnoglucoside, compound *1* was insoluble in 66 % EtOH.

Diosmetin 7-O-[2,4-di-O-(α-L-rhamnopyranosyl)]-β-D-glucopyranoside (1). UV (99.9 % MeOH): 252, 267, 343; (+$AlCl_3$): 265sh, 272, 294sh, 365sh, 384; (+$AlCl_3$/HCl): 264sh, 274, 294sh, 357, 381sh; (+MeONa): 266, 325, 383; (+NaOAc): 258sh, 266, 339; (+NaOAc/H_3BO_3): 254sh, 266, 345 nm. ^{13}C *NMR* (DMSO-d_6): 181.9 (C-4), 164.1 (C-2), 162.4 (C-7), 161.1 (C-5), 157.0 (C-8a), 151.3 (C-4′), 146.8 (C-3′), 122.8 (C-1′), 118.9 (C-6′), 113.1 (C-5′), 112.1 (C-2′), 105.5 (C-4a), 103.9 (C-3), 99.3 (C-6), 94.3 (C-8), 55.8 (-OCH_3), sugar C, see Table 1. R_F values: 0.25 (TBA) and 0.29 (15 % HOAc).

Acid hydrolysis of *1* with 6 % HCl at 100 °C for 3 h gave diosmetin, glucose and rhamnose. The sugars were identified by co-chromatography with authentic samples. *Diosmetin* (4′-*O*-methylluteolin) was identified by UV and chromatographic data. *UV* (99.9 % MeOH): 269, 339; (+$AlCl_3$): 261, 273, 294sh, 361, 387sh; (+$AlCl_3$/HCl): 258, 275, 294sh, 356, 385sh; (+MeONa): 271, 303sh, 374; (+NaOAc): 274, 317sh, 359; (+NaOAc/H_3BO_3): 267, 339 nm. R_F values: 0.76 (TBA) and 0.03 (15 % HOAc). The purple spot viewed in UV did not change colour with NH_3.

Partial hydrolysis of *1* with 6 % HCl at room temperature for 30 days gave, besides diosmetin and *1*, two intermediates *1a* and *1b*. R_F values: *1a* 0.39 (TBA) and 0.27 (15 % HOAc), *1b* 0.26 (TBA) and 0,09 (15 % HOAc).

Enzymatic hydrolysis was carried out with β-glucosidase at 37 °C in an acetate buffer solution (pH 5.0). *1* and *1a* did not hydrolyze but *1b* was rapidly hydrolyzed (< 2 h) to diosmetin.

The permethyl ether of *1* was prepared with NaH, DMSO and CH_3I according to Hakomori's procedure.[10] The permethyl ether was purified by TLC on silica gel with $CHCl_3$ – acetone (3:1) as eluent. *MS* [70 eV; *m/e* (% rel. int.)]: 567 (3), 535 (2), 517 (2), 516 (1), 503 (1), 501 (1), 379 (2), 330 (1), 329 (5), 328 (4), 327 (1), 209 (14), 190 (10), 189 (100), 188 (8), 157 (24), 145 (11), 131 (6), 129 (10), 125 (9), 117 (6), 115 (9), 113 (7), 101 (33), 99 (26), 97 (9), 89 (11), 88 (22), 85 (9), 83 (6), 75 (15), 74 (8), 73 (11), 72 (7), 71 (9), 69 (7), 59 (20), 57 (17), 55 (10). Only peaks larger than 6 % (1 % *m/e* 300 – 900) of the base peak are given.

Linkage analysis of the sugar was performed as described earlier.[9] GLC analysis of the methylated alditolacetates gave 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-rhamnitol (T = 0.47) and 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl-D-glucitol (T = 4.25).

Apigenin 7-O-[2,4-di-O-(α-L-rhamnopyranosyl)]-β-D-glucopyranoside (2). ^{13}C NMR (DMSO-d_6): 181.9 (C-4), 164.4 (C-2), 162.4 (C-7), 162.0 (C-5), 161.2 (C-4′), 157.0 (C-8a), 128.6 (C-2′, C-6′), 120.6 (C-1′), 116.3 (C-3′, C-5′), 105.5 (C-4a), 103.1 (C-3), 99.4 (C-6), 94.4 (C-8), sugar C, see Table 1.

The permethyl ether of *2* was prepared according to Hakomori's method and purified by TLC.[10] *MS* [20 eV; *m/e* (% rel. int.)]: 568 (1), 567 (4), 536 (1), 535 (3), 503 (2), 488 (1), 487 (6), 486 (5), 471 (1), 393 (1), 379 (3), 363 (1), 361 (3), 347 (1), 329 (1), 315 (1), 300 (1), 299 (5), 298 (6), 190 (11), 189 (100), 188 (3), 157

(16), 145 (3), 125 (3), 101 (4), 99 (10), 88 (5), 69 (3), 59 (3). Only peaks larger than 3 % (1 % for m/e 300 – 900) of the base peak are given.

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