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

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

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

The ¹³C NMR spectrum of I in DMSO-$_d_6$
confirmed that it was a glycoside of diosmetin. The
¹³C NMR shifts of the aglycone part of 1 corre-
spend 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 ¹³C NMR spectrum also shows that there
are one glucose and two rhamnose units in I
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.⁵,⁶
For comparison, the ¹³C NMR spectrum of the
earlier isolated apiigenin 7-O-[2,4-di-O-(α-L-
rhamnopyranosyl)]-β-D-glucopyranoside (2) was
recorded. The sugar moieties of the two tri-
glycosides show a very close relationship to
each other, indicating that I has the same link-
ages 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.⁷ Only
peaks from a terminal rhamnose unit, m/e 189,

| Table 1. ¹³C NMR shifts of the sugar moieties ⁴ of the isolated flavonoids. |
|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Compound |
| C-1G | C-2G | C-3G | C-4G | C-5G | C-6G | C-1R | C-2R | C-3R | C-4R | C-5R | C-6R |
| 1    | 100.8 ⁶ | 75.6 | 75.6 | 70.5 | 77.1 | 59.7 | 100.4 ⁶ | 70.5 | 70.5 | 71.9 | 68.7 | 18.2 |
| 2    | 100.8 ⁶ | 75.7 | 75.7 | 70.7 ⁵ | 77.1 | 59.7 | 100.5 ⁶ | 70.5 ⁵ | 70.7 ⁵ | 72.0 | 68.5 | 18.2 |

⁴ G refers to glucose and R to rhamnose. ⁵,⁶ 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-\( \alpha \)-rhamnitol and 1,2,4,5-tetra-O-acetyl-3,6-di-O-methyl-D-glucitol. 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.

![Chemical structure of 1](image)

Considering these data, the structure of 1 is proposed to be diosmetin 7-O-[2,4-di-O-(\( \alpha \)-L-rhamnopyranosyl)]-\( \beta \)-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. 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. Approximately 25 mg of a flavone glycoside (7) 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 % \( \text{EtOH} \).

Diosmetin 7-O-[2,4-di-O-(\( \alpha \)-L-rhamnopyranosyl)]-\( \beta \)-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\(_2\)BO\(_3\)): 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), 98.3 (C-6), 94.3 (C-5), 55.8 (OCH\(_3\)), sugar C, see Table 1. \( R_F \) values: 0.25 (TBA) and 0.29 (16 % 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-methylleuteolin) 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, 305sh, 374; (+ NaOAc): 274, 317sh, 358; (+ NaOAc/H\(_2\)BO\(_3\)): 267, 339 mm. \( R_F \) 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 \( \beta \)-glucosidase at 37 °C in an acetate buffer solution (pH 5.0). 1a and 1b did not hydrolyze but 1b was rapidly hydrolyzed (<2 h) to diosmetin.

**The permethyl ether** of 1 was prepared with NaH, DMSO and CH\(_2\)I according to Hako- mori's procedure. 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 (5), 535 (2), 517 (2), 516 (1), 505 (1), 501 (1), 379 (2), 330 (1), 329 (5), 228 (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. GLC analysis of the methylated alditol acetates gave 1,5-di-O-acetyl-2,3,4,tri-O-methyl-\( \alpha \)-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-(\( \alpha \)-L-rhamnopyranosyl)]-\( \beta \)-D-glucopyranoside (2). \( ^13 \)C NMR (DMSO-\( d_6 \)): 181.9 (C-4), 164.4 (C-2), 162.4 (C-7), 160.2 (C-5), 161.2 (C-4’), 157.0 (C-8a), 128.6 (C-2’), 120.6 (C-1’), 116.3 (C-3’), 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. \( MS \) [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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