Flavone-7-O-sophorotriosides and Biflavonoids from the Moss *Leptostomum macrocarpon* (Leptostomataceae)*

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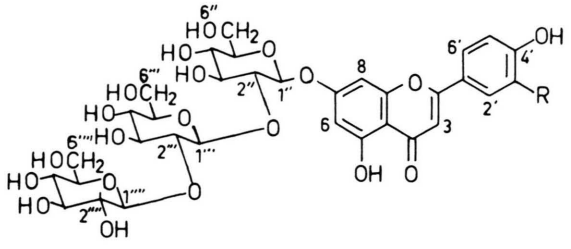
From the gametophytes of *Leptostomum macrocarpon* have been isolated the hitherto unknown 7-O-sophorotriosides of apigenin and luteolin as well as several known biflavonoids. The structures of the new compounds have been proved spectroscopically.

Introduction

The Bryaceae and Mniaceae exhibit the most diverse and complex flavonoid patterns of all moss families that have been studied so far (Geiger *et al.*, 1997). The monogeneric family Leptostomataceae is closely related to Bryaceae and Mniaceae. Thus it seemed promising to investigate the flavonoids of *Leptostomum macrocarpon*, a representative of this family.

Results and Discussion

Seven different flavonoids (**1–7**) were isolated from *L. macrocarpon*. Four of them turned out to



5: R = OH
6: R = OH, acetylated at OH 6", 6"' or 6""
7: R = H

be the known biflavonoids Dicranolomin (**1**), 5', 3'"-Dihydroxyrobustaflavone (**2**), 5', 3'"-Dihydroxyamentoflavone (**3**) and 2, 3-Dihydro-5', 3'"-dihydroxyamentoflavone (**4**); they were identified by their relative molecular mass (FAB-MS), ^{1}H-NMR spectra (see Geiger *et al.*, 1993 and Anhut *et al.*, 1987), and chromatographic comparison with authentic samples (*c. f.* Geiger *et al.*, 1997). The compounds **5-7** are much more hydrophilic, and in the usual TLC systems (see Markham, 1989) they show up in the flavoneglycoside range.

The FAB mass spectrum of the main compound (**5**) shows negative ions at 771 and 285 m/z which suggests that it is a tetrahydroxyflavone trihexoside. Its full structure was elucidated with the aid of its ^{1}H- and ^{13}C-NMR spectra, which are presented in Table I. In the ^{1}H-NMR spectrum multiplicity and chemical shift of the signals identify readily the aglycone protons of a luteolin-7-glycoside and three anomeric sugar protons (see Markham and Geiger, 1994). The ^{13}C-NMR spectrum shows 15 signals attributable to the aglycone and 18 signals that must be attributed to the sugar moiety. By one- and multible-bond correlation of the ^{1}H and ^{13}C-NMR all signals can be assigned. This reveals that both interglycosidic linkages between the three hexoses are 1→2. By comparing the chemical shifts of the ^{13}C resonances with published data of various flavonoid mono-, di- and triglycosides (*c. f.* Markham *et al.*, 1982) it can be demonstrated that all three hexoses are glucose. Thus **5** is luteolin-7-ß-sophorotrioside.

The second glycoside (**6**) seemed to be an ester of **5** since it is easily hydrolysed to **5**. Therefore only a very small amount could be isolated in a pure state. This was just enough to run the FAB mass spectrum and a ^{1}H-NMR spectrum (see Table II). The mass spectrum revealed that the rel-

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Table I. NMR data of luteolin-7-sophorotrioside (**5**).

C / H No	^{1}H	^{13}C	C,H-long-range couplings recorded by the "inverse" technique at 500 MHz
2		164.7	
3	6.71 s	102.4	C-2, C-4, C-10, C-1'
4		181.6	
5		161.0	
6	6.47 d (1.7)	99.4	C-5, C-7, C-8, C-10
7		162.5	
8	6.83 d^m	94.6	C-6, C-7, C-9, C-10
9		156.8	
10		105.2	
1'		119.8	
2'	7.39 d^m	112.8	C-2, C-3', C-4', C-6'
3'		146.3	
4'		152.0	
5'	6.84 d (8.4)	115.9	C-3, C-1', C-3', C-4'
6'	7.41 dd (1.9/8.4)	119.2	C-2, C-2', C-4'
1"	5.22 d (7.5)	98.2	C-7
2"	3.45 m	82.9	C-1", C-3", C-1"'
3"	3.58 m	75.4	C-2", C-4"
4"	3.25 m	68.6	C-6"
5"	3.20 m	76.0	
6"	3.44/3.34 m m	60.2	
1"'	4.61 d (7.7)	102.2	C-2"
2"'	3.25 m	83.1	C-1"', C-1""
3"'	3.45 m	76.0	C-2"', C-4"'
4"'	3.22 m	69.1	
5"'	3.48 m	76.9	C-1"', C-3"'
6"'	3.71/3.47 m m	60.5	
1""	4.51 d (7.7)	104.1	C-2"', C-2""
2""	3.03 m	74.6	C-1""
3""	3.16 m	76.3	C-4""
4""	3.07 m	69.7	C-5"", C-6""
5""	3.48 m	77.2	C-1"', C-3""
6""	3.74/3.50 m m	60.9	C-4""

Table II. ^{1}H-NMR-data of the acetylated luteolin-7-sophorotrioside (**6**).

H	^{1}H
3	6.74 s
6	6.41 d (2)
8	6.82 d (2)
2'	7.61 d (2)
5'	6.83 d (7.5)
6'	7.39 dd (2/7.5)
1"	5.16 d (7.5)
1"'	4.71 d (8)
1""	4.48 d (8)
6_a*)	4.32 d (11)
6_b*)	3.94 dd (6.5/11)
other glc-H	3.0–3.8 m
acetyl	1.74 s

*) H-6_a and 6_b of the acetylated glucose moiety.

ative molecular mass is 42 units higher than that of **5**; this hints to acetic acid as the acid component of the ester **6**. A three-proton singlet at 1.74 ppm in the ^{1}H-NMR spectrum confirms the assumption that **6** is an acetate. Two signals centered at 4.32 ppm and 3.94 ppm point to the site of acetylation. On the basis of their coupling patterns and chemical shifts these signals must be assigned to the two nonequivalent protons at the C-6 of a 6-O-acetylglucose. However, on the basis of the available evidence one cannot decide which glucose moiety is acetylated. Thus it can only be said that **6** is a luteolin-7-O-ß-[(6" or 6"' or 6"")-O-acetyl]-sophorotrioside.

The structure of the third glycoside (**7**) can be deduced easily from its ^{1}H and ^{13}CNMR spectra (see Table III): within the limits of experimental error the sugar signals are identical with those of **5** and the aglycone signals agree with the data of apigenin-7-glycosides (*c. f.* Markham and Geiger, 1994 and Markham *et al.*, 1982). Thus **7** is apigenin-7-O-ß-sophoroside.

Table III. NMR data of the apigenin-7-sophorotrioside (**7**).

H / C	^{1}H	^{13}C
2		164.2
3	6.83 s	102.8
4		181.8
5		161.6
6	6.48 d (2.0)	99.4
7		162.8
8	6.87 d (2.0)	94.8
9		156.8
10		105.2
1'		120.6
2'	7.93 d (8.8)	128.4
3'	6.92 d (8.8)	116.0
4'		160.9
5'	6.92 d (8.8)	116.0
6'	7.93 d (8.8)	128.4
1"	5.21 d (7.6)	98.2
2"	3.0–3.8 m	82.8
3"	" " "	75.4
4"	" " "	68.6
5"	" " "	76.0
6"	" " "	60.2
1"'	4.62 d (7.7)	102.2
2"'	3.0–3.8 m	83.1
3"'	" " "	76.3
4"'	" " "	69.1
5"'	" " "	76.9
6"'	" " "	60.4
1""	4.51 d (7.7)	104.1
2""	3.0–3.8 m	74.5
3""	" " "	77.2
4""	" " "	69.7
5""	" " "	78.3
6""	" " "	60.9

These three flavone sophorotriosides are to the best of our knowledge new natural compounds, whereas the biflavonoids of *L. macrocarpon* are also widespread in the neighbouring families Bryaceae and Mniaceae. This fits well into the general picture of the distribution of flavonoids in arthrodontous mosses: biflavonoids provide rarely useful characters that separate taxa below the rank of the order or suborder, but flavonoid glycosides, if they are present at all, usually characterize genera or even subgenera (Geiger *et al.*, 1997).

Experimental

Plant material. Gametophytes of *Leptostomum macrocarpon* (Hedw.) Pyl. was collected in February 1991 at Nikau-grove near Pika, North Island, New Zealand. A voucher is deposited in the private herbarium of H. G. (No. 1804). Only that portion of the material was used, which was presumably living at the time of collection.

Extraction and isolation. The airdried material was exhaustively extracted by repeated maceration with MeOH/H_2O (4:1) containing 1% citric acid. Elimination of lipids and chlorophyll from the combined extracts and preliminary separation by MPLC on RP-18 with a H_2O/MeOH gradient ranging from 9:1 to 2:2 was performed as described earlier (Rampendahl *et al.*, 1996). From this column the flavonoids were eluted in the sequence **5** + **6**, **7**, **1**, **2** + **3** + **4**. Further separation and removal of non-flavonoid matter was achieved by SC on Sephadex LH20 using as eluents MeOH/H_2O (20–50% for sepn of **5** and **6** and 80% for general purification) and Me_2CO/MeOH/H_2O (2:1:1) for sepn of **2**, **3** and **4**. By these methods we isolated from 12 g *L. macrocarpon* in pure state 5 mg **1**, 6 mg **2**, 12 mg **3**, 29 mg **5**, 1,5 mg **6** and 5 mg **7**, as well as 2 mg **4** containing ~ 25% **3**.

FAB-MS (neg. mode): 4–7 keV Xe and glycerol as matrix. NMR, unless stated otherwise on the Tables: DMSO-d_6, ambient temperature, 400 MHz (^{1}H) and 100 MHz (^{13}C).

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