Triflavones and a Biflavone from the Moss *Rhizogonium distichum*

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Dedicated to Professor Hans Dietmar Zinsmeister on the occasion of his retirement

Rhizogonium distichum, Dicranolomin, Triluteolins

From gametophytes of *Rhizogonium distichum* have been isolated the biflavone dicranolomin and five triluteolins. Two of the triluteolins, which were named rhizogoniumtriluteolin and distichumtriluteolin, were new compounds; their structures have been elucidated spectroscopically.

Introduction

In the course of a recent chromatographic study on the occurrence of flavonoids in arthrodontous mosses (*c.f.* Geiger *et al.*, 1997) it has been observed that *R. distichum* is an extremely rich source of bi- and triflavones. It seemed therefore promising to study this species in more detail.

Results and Discussion

By column-chromatography with our standard systems (see Seeger *et al.*, 1992, 1993, 1995) an aqueous-acetonic extract of *Rhizogonium distichum* was separated into five fractions, three of which proved to consist of only one compound each (**1, 4** and **5**), whereas the other two were according to their ^{1}H NMR spectra mixtures containing the known triluteolins **2a, 2b** and **3**. The spectra of these two mixtures allowed to analyse them qualitatively and – with the help of the integrals of some well separated signals – even semiquantitatively. Therefore the tedious isolation of pure **2a, 2b** and **3**, which has been described by Seeger *et al.* (1995) was not repeated.

The first pure compound could be identified by its NMR spectra as dicranolomin (**1**) (*c.f.* Geiger *et al.*, 1993). The two unknown compounds **4** and **5** showed spots on TLC plates whose appearance under UV-light (untreated dark and fluorescing yellow after spraying with diphenylboric acid β-aminoethylester) was reminescent of luteolin and its di- and trimers (Geiger *et al.*, 1997). The LAMMA mass spectra of both compounds exhibit [M-1] anions at 853 *m/z*. These facts suggest that they are both triluteolins, isomeric with **2a, 2b** and **3** (*c.f.* Seeger *et al.*, 1995). The ^{1}H NMR spectra of **4** and **5** exhibit, like the spectrum of **3**, a doubling of most signals. This reveals that both consist, like **3**, of a pair of equilibrating rotamers. The ratio of the two rotamers, however, was in both cases almost 1:1. This meant that the two sets of proton signals could not be separated on the basis of their integrals and one had to resort to various correlation techniques. With compound **4**, which shall be named rhizogoniumtriluteolin, most of the proton signals could be assigned to one or the other rotamers. The results are presented on Table I. Since the effects that cause the doubling of most proton signals have little influence upon the chemical shift of the carbon signals a comparison of the ^{1}H–^{13}C correlation spectrum with the spectra of various biflavonoids (*c.f.* Table II and Geiger *et al.*, 1993) allowed to assign every proton its position at one of the three luteolin skeletons. The sets of coupling protons were disentangled by COSY. A ROESY spectrum was recorded in order to see possible NOE interactions of I H-3 with I H-6′ and II OH-5, II H-3 with II H-6′ as well as III H-3 with III H-2′ and III H-6′. Quite unexpectedly this spectrum showed also within each luteolin moiety correlations between OH-5 and H-3 as well as H-6. These correlations cannot be explained in terms of NOE effects since the chemical shift of OH-5 in 5-hydroxyflavones (usually about 12–13 ppm) indicates that this proton forms a hydrogen bond with the carbonyl group. These unknown ef-

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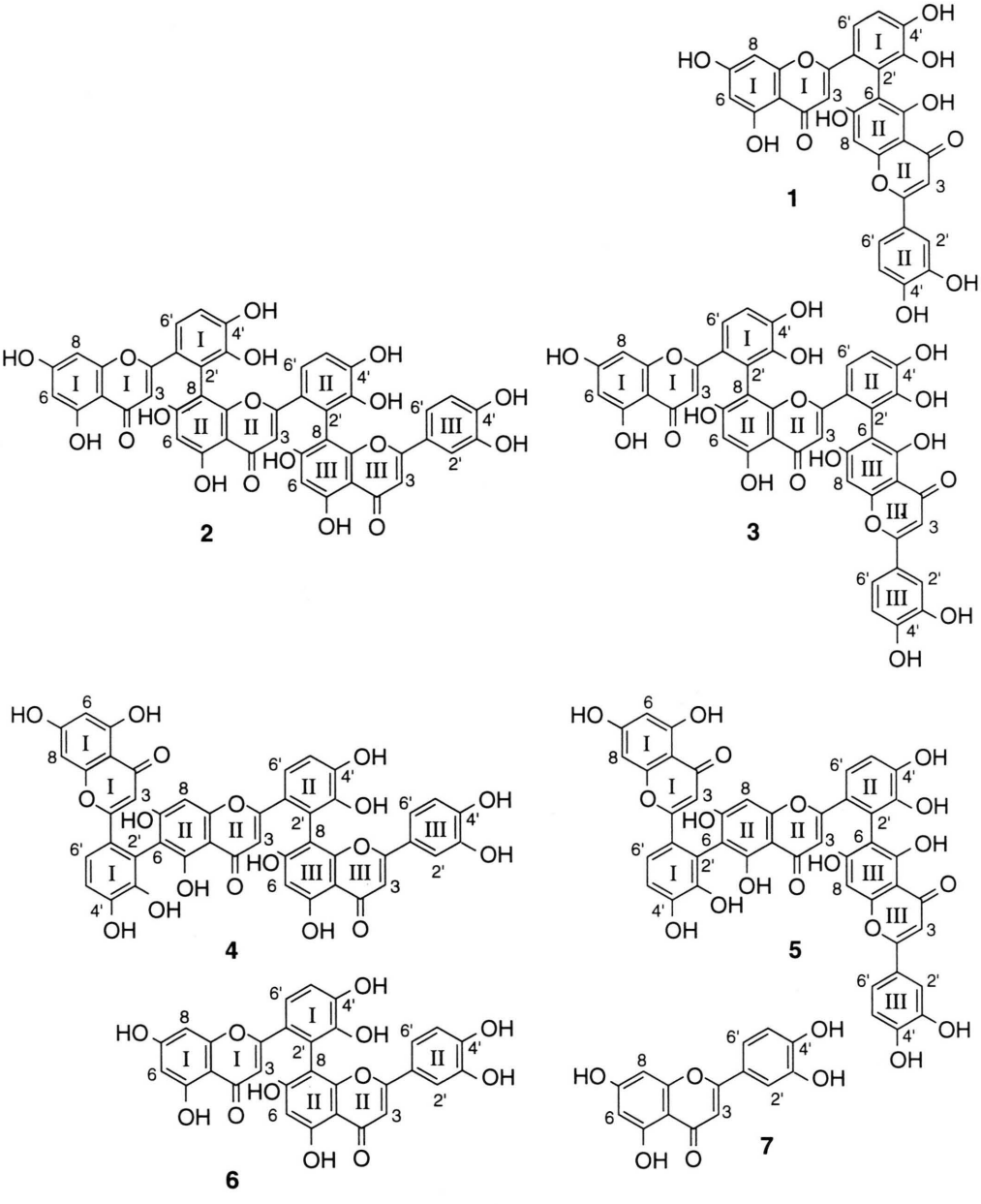

Fig. 1. Planar structures and numbering system of dicranolomin (**1**), the two diastereomers bartramiatriluteolin (**2a**) and epibartramiatriluteolin (**2b**), strictatriluteolin (**3**), rhizogoniumtriluteolin (**4**), distichumtriluteolin (**5**), philonotisflavone (**6**), and luteolin (**7**).

fects are also observed in the ROESY spectrum of luteolin itself, if it is recorded with the same "mixing time" (250 msec); therefore these correlations were also used to analyse the spectra. The only well spaced proton signals that could not be assigned to one or the other of the two isomers were the signals of the protons at position II-8, which are too distant from any other proton. If one com-

Table I. NMR data of the triflavones **4** and **5**.

Luteolin unit	Position	Rhizogoniumtriluteolin (**4**) ^1H a	^1H b	^{13}C a/b	Distichumtriluteolin (**5**) $^1H^\dagger$	$^1H^\dagger$	^{13}C
	3	5.94 s	5.87 s	106	5.96 s	5.93 s	106
	5-OH	12.75 s	12.70 s	–	12.76 s	12.75 s	–
	6	6.07 d^m	6.05 d^m	98	6.09 d^m	6.08 d^m	98
I	8	5.96 br s	5.96 br s	93	5.98 br s	5.97 br s	93
	5′	6.91 d^o	6.90 d^o	114	6.91 d^o	6.90 d^o	114
	6′	7.15 d^o	7.13 d^o	120.5	7.15 d^o	7.13 d^o	120
	3	6.10 s	5.89 s	106	6.05 s	5.93 s	106
	5-OH	13.00 s	12.91 s	–	12.98 s	12.93 s	–
	6	–	–	107.5	–	–	107.5
II	8	5.86 s*	6.15 s*	93	6.25 s	6.07 s	93
	5′	6.99–7.06 m	6.99–7.06 m	114	6.96 d^o	6.95 d^o	114
	6′	7.25 d^o	7.27 d^o	120	7.23 d^o	7.25 d^o	120
	3	6.62 s	6.60 s	102	6.69 s	6.69 s	103
	5-OH	13.04 s	13.03 s	–	13.21 s	13.17 s	–
III	6	6.23 s	6.28 s	98	–	–	107.5
	8	–	–	103.5	6.55 s	6.51 s	93
	2′	6.99–7.06 m	6.99–7.06 m	113.5	7.41 d^m	7.41 d^m	113
	5′	6.73 d^o	6.73 d^o	115	6.88 d^o	6.88 d^o	116
	6′	6.99–7.06 m	6.99–7.06 m	118.5	7.42 dd^{om}	7.42 dd^{om}	118.5

* These signals cannot be assigned to a or b.
† Neither singlets nor sets of coupled signals can be assigned to specific rotamers.

Table II. NMR data of the biflavones **1** and **6**. Correlated ^{13}C and ^1H data taken from Geiger *et al.* (1993) and data of the OH-5 protons as well as the diagnostically important quaternary carbons from Seeger (1992).

Luteolin unit	position	Dicranolomin (**1**) ^1H	^{13}C	Philonotisflavone (**6**) ^1H	^{13}C
	3	6.04 s	106.2	6.03 s	106.4
	5-OH	12.78 s	–	12.73 s	–
	6	6.08 d^m	98.6	6.07 d^m	98.6
I	8	5.97 d^m	93.3	5.76 d^m	93.0
	5′	6.94 d^o	114.6	7.01 d^o	114.6
	6′	7.17 d^o	120.5	7.24 d^o	120.5
	3	6.65 s	103.3	6.57 s	102.4
	5-OH	13.13 s	–	13.01 s	–
	6	–	107.9	6.27 s	98.3
II	8	6.53 s	93.1	–	103.5
	2′	7.40 d^m	113.3	6.89 d^m	113.5
	5′	6.90 d^o	116.0	6.74 d^o	115.5
	6′	7.41 dd^{om}	118.9	6.89 dd^{om}	118.9

pares the NMR data of **4** with those of **1** and **6**, which are shown on Table II, it is obvious that the signals that are assigned to the luteolin moiety I are almost the same as with the luteolin moiety I of dicranolomin (**1**), whereas the signals of the luteolin moiety III resemble the moiety II of philonotisflavone (**6**). Thus the two terminal moieties I and III are linked to the central luteolin II *via* their carbons 2′ and 8, respectively. Multiplicity and chemical shift of the signals attributed to luteolin II indicate that it is linked *via* its carbons 6 and 2′. The NOE interaction between I H-3 and II OH-5 indicates clearly that the interflavonyl-linkages within **4** are I 2′→II 6 and II 2′→III 8; this is corroborated by the presence of a II-C-6 signal at 107.5 ppm which is clear of the range of other carbon signals (*c.f.* Seeger *et al.*, 1995).

The other unknown triluteolin shall be named, after the specific epithet of *R. distichum*, distichumtriluteolin (**5**). The spectra of this compound were analysed by the same techniques, which are described above with compound **4**. This led also to unquestionable assignements of every resonance to the position of the respective nuclei within the triluteolin skeleton (see Table I). In contrast to the case of rhizogoniumtriluteolin (**4**),

however, it was not possible to separate with confidence the spectra of the two rotamers of distichumtriluteolin (**5**), because the differences between the two spectra were too small. If the NMR data of **5** are compared with those of dicranolomin (**1**) on Table II, it can be seen that the signals assigned to the luteolin units I and III of **5** are near identical with the moieties I and II of **1**, respectively. This confirms the sites of the interflavonyl linkages at the luteolins I and III. Finally, the close resemblance of the signals attributed to the luteolin units II of **4** and **5** suggests that the interflavonoyl linkages of **5** are I 2′→II 6 and II 2′→III 6. This is confirmed by the observation of NOE interactions between I H-3 and II OH-5 as well as II H-3 and III OH-5, and the presence of carbon resonances at about 107.5 ppm, which must be attributed to II C-6 and III C-6 (see above and Seeger *et al.*, 1995).

The combined yields of the flavonoids **1–5** that were isolated from only 2.8 g of dried *R. distichum* amounted 276 mg, which is almost 10% (!). This unusually high concentration of bi- and triflavones in *R. distichum* raises the question why this plant should spend such a large proportion of its metabolic resources to synthesize them. Two possible functions of these compounds are discussed at present: 1st protection from UVB-light and 2nd protection from noxious organisms (*c.f.* Geiger *et al.*, 1997). If one considers the usual habitat of this little moss on rotting wood in wet forests, the second function seems more likely, namely the protection from the attack of degrading enzymes that are produced by the organisms, which cause the rotting of wood.

Experimental

Rhizogonium distichum (Sw.) Brid. (2.8 g dry gametophytes) was collected with permission of the Department of Conservation in spring 1994 by M. Veit at about 900 m.s.m. in Egmont National Park, New Zealand. A voucher is kept in the private herbarium of H. G. (Nr. 290 R).

The plant material was exhaustively extracted by two-day mazerations with Me_2CO-H_2O (4:1 v/v). The extract was worked up according to Seeger *et al.* (1992, 1993 and 1995) by CC with polyamide-6 and a H_2O-Me_2CO gradient and Sephadex LH 20 with the eluents $Me_2CO/MeOH/H_2O$ (2:1:1 v/v/v) and Me_2CO/H_2O (4:1 v/v). With these systems the sequences of elution were **1, 2a, 3, 2b, 4+5; 2a, 3, 2b, 4, 1, 5;** and **2a, 3, 2b, 1, 4, 5** respectively. The yields were 111 mg **1**, 25 mg **4**, 38 mg **5**, 86 mg **2a+3** (2:1), and 16 mg **2a+2b+3** (1:3:4).

M_r of **4** and **5** were determined by laser induced microprobe mass analysis (LAMMA) with nicotinic acid amide as matrix.

NMR spectra were recorded at ambient temperature in DMSO-d_6 with 500 (^{1}H) and 250 (^{13}C) MHz. The ROESY spectra were recorded using a mixing time of 250 msec.

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