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THE FIRST BIAURONE, A TRIFLAVONE AND BIFLAVONOIDS FROM TWO *AULACOMNIUM* SPECIES*

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Key Word Index—*Aulacomnium palustre*; *A. androgynum*; Aulacomniaceae; Musci; aulacomniumbiaureusidin; aulacomniumtriluteolin; biflavones; dihydrobiflavones; ^{1}H NMR; ^{13}C NMR.

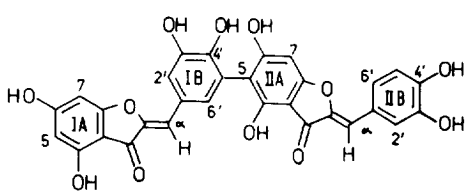
Abstract—The flavonoid pattern of two *Aulacomnium* spp. has been studied. Besides seven known biflavonoids the new aulacomniumbiaureusidin and aulacomniumtriluteolin have been isolated; their structures have been established by spectroscopic methods.

INTRODUCTION

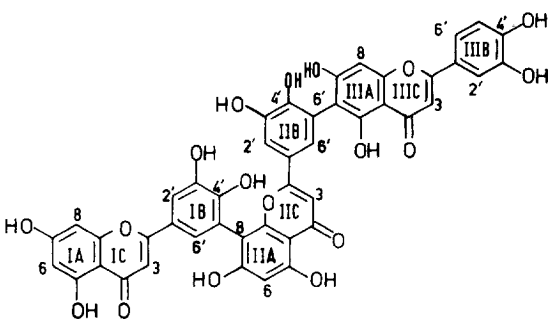
A chromatographic survey of the Aulacomniaceae has shown that species of this moss family contain some hitherto unknown flavonoids, in addition to known biflavonoids [1]. This prompted us to study this family in more detail. According to a recent treatment the Aulacomniaceae comprise only the one genus *Aulacomnium* [2]. Two species—*A. palustre* and *A. androgynum*—were available in sufficient quantities to permit structure investigations of the unknown flavonoids.

RESULTS AND DISCUSSION

The work-up of an aqueous acetone extract of *A. palustre* yielded two new flavonoids, aulacomniumbiaureusidin (**1**) and aulacomniumtriluteolin (**2**), together with four known biflavonoids. The UV spectrum of **1** (see Table 1), an $[M - H]^-$ ion at 569 *m/z* in the FAB-MS, and the appearance in UV light (bright green, turning orange after spraying with diphenylboric acid β-aminoethyl ester) suggested that **1** is a biaureusidin.



1



2

This was confirmed by the ^{13}C NMR spectrum (Table 2). In this spectrum, the signals associated with aureusidin-I are nearly identical with those of the aureusidin moiety in campylopusaurone, which is C–C linked at the IB-5′ position to an eriodictyol unit [3]. It is thus evident that aureusidin-I in **1** is linked at the IB-5′ position to aureusidin-II. The carbon resonances of aureusidin-II (Table 2) match with those of aureusidin itself, except for the carbons IIA4, 5 and 6, which differed by − 1.2, + 11.0 and − 2.3 ppm, respectively. These data require that the interflavonyl linkage point on aureusidin-II be at carbon-IIA-5 [4]. The interflavonyl linkage in **1** is therefore defined as IB-5′ → IIA-5. This is supported also by the ^{1}H NMR data, which have already been published in another context [5].

The chromatographic characteristics and UV spectrum (Table 1) of **2** closely resemble those of luteolin and its dimers [6]. A $[M - H]^-$ ion at 853 *m/z* in the FAB mass spectrum, however, indicates that **2** is a triluteolin. The sites of the two interflavonyl linkages are readily deduced from the NMR spectra (Table 3). In the ^{1}H NMR spectrum the three protons of the IIIB-ring are easily identified by their characteristic coupling pattern. Their chemical shifts indicate that they are not shielded

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by an aryl substituent at position 8 of the IIIA-ring [5]. Thus, the luteolin unit III is linked at the IIIA6 position to the middle luteolin unit II, and the singlet at 6.24 ppm, which is correlated with a carbon signal at 93.2 ppm, represents H-IIIA8. Two pairs of *meta*-coupled protons (7.13/7.10 and 7.30/7.46 ppm) prove that the two other luteolin units (I and II) are linked via their B5′ carbon atoms. The second bridgehead of the middle luteolin (II) must be at position IIA-8. This is demonstrated by the strong shielding of H-IIB2′ and H-IIB6′ doublets at 7.13 and 7.10 ppm, respectively, and by the presence of the H-IIB6 singlet at 6.32 ppm, which is correlated with the C-IIB6 signal at 99.1 ppm [5]. The remaining proton doublets are as expected for the IA-ring. All carbon signals are also in accordance with the proposed structure of **2**.

Table 1. UV absorption spectra of **1** and **2**

Solvent and reagents	**1**	**2**
MeOH	265, 325*sh*, 411	268*sh*, 346
+ NaOMe	460	270*sh*, 406
+ $AlCl_3$	268*sh*, 349, 526	275, 425
+ $AlCl_3$/HCl	327*sh*, 406, 469*sh*	276*sh*, 361, 388*sh*
+ NaOAc	277*sh*, 344, 432	269, 375
+ NaOAc/H_3BO_3	279*sh*, 337, 433	263, 376

The known biflavonoids—5′,3‴-dihydroxyamentoflavone (**3**), 5′,3‴-dihydroxyrobustaflavone (**4**), 2,3-dihydro-5′,3‴-dihydroxyamentoflavone (**5**) and dicranolomin (**6**)—were identified by comparison of their NMR spectra with published data [5, 7].

A. androgynum differs from *A. palustre* in that it lacks the triflavone **2**, but contains the biflavonoids philonotis-

Table 2. ^{13}C and ^{1}H NMR data for **1** and **9**, recorded in DMSO-d_6 at ambient temperature (125.500 MHz and 100/400 MHz, respectively). Quarternary C signals identified by DEPT and assigned by 'best fit'. Inverse C–H correlated signals of **1** taken from *l.c.* [5]. Proton signals of **9** assigned on the basis of a ^{1}H–^{1}H-COSY experiment and their chemical shift

		1			**9**	
	No.	^{13}C	^{1}H	No.	^{13}C	^{1}H
	x	109.9	6.45 *s*	**2**	76.9	4.95 *dd* (3; 13)
	2	145.5		**3**	42.7	ax 3.08 *dd* (13; 17)
						eq 2.51 *dd* (3; 17)
	3	179.0		**4**	196.1	—
	4	166.8		**5**	163.2	—
	5	97.6	6.06 d^m	**6**	95.6	5.80 *s*
	6	167.4		**7**	166.4	—
I	**7**	90.2	6.15 d^m	**8**	94.7	5.80 *s*
	8	158.1		**9**	163.0	—
	9	102.9		**10**	101.5	
	1′	122.4		**1′**	129.3	—
	2′	115.8	7.41 d^m	**2′**	118.8	—
	3′	145.7	—	**3′**	143.2	—
	4′	147.5		**4′**	144.9	—
	5′	120.8		**5′**	114.5	6.88 d^o
	6′	127.3	7.03 d^m	**6′**	116.0	6.95 d^o
	x	109.5	6.50 *s*	**2**	163.7	—
	2	145.9		**3**	102.9	6.67 *s*
	3	180.1		**4**	181.6	—
	4	165.8		**5**	159.1	—
	5	108.7		**6**	106.7	—
	6	165.2		**7**	161.8	—
	7	90.5	6.36 *s*	**8**	93.3	6.54 *s*
II	**8**	158.1		**9**	156.3	—
	9	102.9		**10**	102.8	—
	1′	123.7	—	**1′**	121.6	—
	2′	117.5	7.44 d^m	**2′**	113.2	7.39 d^m
	3′	145.5		**3′**	145.6	—
	4′	147.5	—	**4′**	149.5	—
	5′	115.8	6.83 d^o	**5′**	116.3	6.86 d^o
	6′	124.0	7.20 dd^{om}	**6′**	118.9	7.41 dd^{om}

Table 3. ^{13}C and ^{1}H NMR data for **2**, recorded in DMSO-d_6 at ambient temperature (100/400 MHz, respectively). Quarternary C signals assigned by 'best fit'. ^{1}H and ^{13}C–^{1}H signals identified on the basis of ^{1}H–^{1}H and 'inverse' detected ^{1}H–^{13}C correlations

No.	I	I	II	II	III	III
2	163.3	—	163.6	—	163.6	—
3	102.7	6.43 *s*	102.2	6.35 *s* (*br*)	102.4	6.53 *s*
4	181.8	—	181.3	—	181.1	—
5	161.4	—	158.8	—	161.4	—
6	98.5	5.96 d^m	99.1	6.32 *s*	108.3	—
7	164.6		161.2	—	162.3	—
8	93.5	6.16 d^m	104.9		93.2	6.24 *s* (*br*)
9	156.9		154.6		155.9	—
10	103.4	—	103.3	—	102.9	—
1′	120.3		121.7		121.1	—
2′	111.3	7.30 d^m	111.6	7.13 d^m	113.5	7.36 d^m
3′	145.5		146.1	—	145.6	—
4′	149.1		149.2	—	149.3	—
5′	119.9		120.2	—	115.9	6.92 d^o
6′	122.0	7.46 d^m	122.5	7.10 (d^m)*br*	118.7	7.34 dd^{om}

flavone (**7**), 2,3-dihydrophilonotisflavone (**8**) and 2,3-dihydrodicranolomin (**9**). The compounds **7** and **8** exhibited ^{1}H and ^{13}C NMR spectra, which agreed with the data given in ref. [8].

Compound **9** has been isolated only once before from *Dicranoloma robustum* in a small amount, which yielded only a ^{1}H NMR spectrum [6]. In the present work **9** was isolated in an amount sufficient to record a ^{13}C NMR spectrum, that is presented in Table 2. The ^{1}H NMR data for **9** from *A. androgynum* are also included in Table 2, because it shows also the signals of the methylene protons at position 3, which were obscured in the spectrum of the *D. robustum* compound. Apart from this, the two spectra are identical within the limits of experimental error.

EXPERIMENTAL

Plant material. Gametophytes of *A. palustre* (Hedw.) Schwaegr. were collected at three sites in Norway, France and Germany. After it had been demonstrated that the three collections were chromatographically identical, they were worked up together. Voucher specimens are deposited at SAAR (Nos 3985, 3986 and 3987). Gametophytes of *A. androgynum* (Hedw.) Schwaegr. were collected near Waldmohr, Rheinland-Pfalz, Germany, Voucher at SAAR No. 3988.

Extraction and isolation. The extraction and preliminary separation by MPLC on a RP-18 support was performed as described *l.c.* [9]. The MPLC of the extract from 500 g (dry wt) *A. palustre* yielded two fractions containing **1** + **2** + **6** and **3** + **4** + **5**, respectively. Further separation was achieved by CC on Sephadex LH20 with a gradient ranging from 50% to 80% MeOH in H_2O. Yields: 18 mg **1**, 16 mg **2**, 120 mg **3**, 90 mg **4**, 9 mg **5** and 4 mg **6**. MPLC of the extract from 180 g dry wt. *A. androgynum* also gave two fractions containing **1** + **6** + **7** + **8** + **9** and **3** + **4** + **5**, respectively. CC on Sephadex with a H_2O–MeOH gradient, as described above, separated the first fraction only in 3 fractions containing **7** + **8**, **6** + **9** and **1**, respectively, whereas the second fraction was resolved completely into **5**, **3** and **4**. The pairs **7** + **8** and **6** + **9** were finally separated by MPLC on RP-18 with 45% MeOH containing 0.5% formic acid. The yields from *A. androgynum* were 3 mg **1**, 24 mg **3**, 30 mg **4**, 6 mg **5**, 25 mg **6**, 4 mg **7**, 11 mg **8** and 12 mg **9**.

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REFERENCES

1. Hahn, H. (1993) Dissertation, Saarbrücken, Germany.
2. Vitt, D. H. (1984) *New Manual of Bryology* 2 (Schuster, R. M., ed.), p. 696. Hattori Botanical Laboratory, Nichinan, Japan.
3. Geiger, H. and Markham, K. R. (1992) *Phytochemistry* **31**, 4325.

4. Markham, K. R., Sheppard, C. and Geiger, H. (1987) *Phytochemistry* **26**, 3335.
5. Geiger, H., Seeger, T., Hahn, H., Zinsmeister, H. D., Markham, K. R. and Wong, H. (1993) *Z. Naturforsch.* **48c**, 821.
6. Markham, K. R., Andersen, Ø. M. and Viotto, E. S. (1988) *Phytochemistry* **27**, 1745.
7. Anhut, S., Seeger, T., Zinsmeister, H. D. and Geiger, H. (1989) *Z. Naturforsch.* **44c**, 189.
8. Geiger, H. and Bokel, M. (1989) *Z. Naturforsch.* **44c**, 559.
9. Salm, R., Seeger, T. and Zinsmeister, H. D. (1993) *Z. Naturforsch.* **48c**, 531.