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TRI- AND TETRAHYDROXYCOUMARIN DERIVATIVES FROM *TETRAPHIS PELLUCIDA**

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Key Word Index—*Tetraphis pellucida*; Tetraphidaceae; Musci; 5,7,8-trihydroxycoumarin derivatives; 5,6,7,8-tetrahydroxycoumarin derivatives.

Abstract—From gametophytes of *Tetraphis pellucida* have been isolated eight different tri- and tetrahydroxycoumarin derivatives; three of them are new natural products, the structures of which were elucidated spectroscopically. The taxonomic relevance of the results is discussed.

INTRODUCTION

In a previous publication it was reported that the main phenolic constituents of two representative species (*Atrichum undulatum* and *Polytrichum formosum*) of the moss family Polytrichaceae are tri- and tetrahydroxycoumarin derivatives [1]. The present paper deals with the isolation of such compounds from *Tetraphis pellucida*. This species was studied because the taxonomic position of the Tetraphidaceae near the Polytrichaceae is still debated [2].

RESULTS AND DISCUSSION

From an aqueous methanolic extract of *T. pellucida*, eight coumarin glycosides (**1**–**8**) were isolated (Table 1). Five of them (**1**, **2**, **4**, **6** and **7**) were identified as known coumarins from Polytrichaceae on the basis of their spectroscopic (UV and ^{1}H and ^{13}C NMR) and chromatographic data [1]. The other three coumarins (**3**, **5** and **8**) were new natural products.

The ^{13}C NMR spectrum of **3** (Table 2) exhibited in the aliphatic range six signals attributable to a β-glucopyranoside moiety [3] and the signal of an aromatic methoxyl group. The aromatic signals were almost superimposable on those of 7-methoxy-5,6,8-trihydroxycoumarin-5-β(6-*O*-malonyl-glucopyranoside), a constituent of *Polytrichum formosum* [1]. This suggested that **3** was 7-methoxy-5,6,8-trihydroxycoumarin-5-β-glucopyranoside. This structure was confirmed by the other spectroscopic data. The FAB-mass spectrum yielded the required M_rs of **3** (386) and its aglycon (224). The 5-position of the glucose moiety was demonstrated by a NOE interaction between the anomeric proton and H-4. The absence of an $AlCl_3$-shift in the UV spectra (Table 1) proved the absence of two neighbouring phenolic hydroxyl groups, therefore the methoxyl group had to be at position 7. The ^{1}H NMR data (Table 3) were also in agreement with the proposed structure of **3**.

Compound **5**, like **3**, appeared to be closely related to a substance which also occurs in Polytrichaceae i.e. the 6′-*O*-malonylester of the 5,7,8-trihydroxycoumarin-5-β-glucopyranoside (**4**). This was evident from its FAB-mass spectrum ($[M-1]^- = 441$), ^{13}C and ^{1}H NMR spectra (Tables 2 and 3) (aglycone signals almost identical with those of **4** [1], and the signals of a 6-*O*-malonylglucose moiety very close to those of other 6-*O*-malonyl-β-glucopyranosides [1, 4]), and a strong $AlCl_3$-shift in the UV spectra (Table 1).

Compound **8** gave rise to a ^{13}C NMR spectrum (Table 2), that showed 12 signals in the sugar region which could be assigned by comparison with published data [3] to a β-sophorosyl moiety, one methoxyl signal and nine signals in the aromatic range. Together with the FAB-mass spectrum ($[M-1]^- = m/z$ 531 and $[M-\text{sophorose}-1]^- = m/z$ 207) this suggested that **8** was the monomethyl ether of a trihydroxycoumarin-β-sophoroside. The location of the substituents was deduced from the ^{1}H NMR spectrum (Table 3) and two NOE-experiments. Two doublets at δ6.15 and 8.44 (J = 9.7 Hz) and a singlet at δ6.90 demonstrated that the lactone ring was unsubstituted and that the carbocycle was trisubstituted. Irradiation at δ8.44 (H-4) enhanced the methoxy signal, thus the methoxyl group had to be at position 5. Irradiation of the singlet at δ6.90 enhanced the signals of the methoxyl group and the anomeric proton at δ4.94, therefore, the proton giving rise to this singlet had to be linked to C-6 and the sophorose must be located at position 7. Thus **8** is 7,8-dihydroxy-5-methoxycoumarin-7-β-sophoroside.

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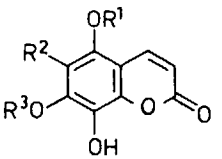
Table 1. Substitution pattern and UV and chromatographic data of the coumarin glycosides from *T. pellucida*



Compound	Substitution pattern			λ_{max} (nm)		TLC (cellulose) Appearance under UV		TLC (cellulose) hR_f-values		HPLC
	R^1	R^2	R^3	MeOH	$AlCl_3$	Untr.	NA	15% HOAc	BAW	R_t (min)
5,6,7,8-Tetrahydroxycoumarin-5-β-glucopyranoside (**1**)	glc	OH	H	257sh; 327	270sh; 366	pg	t	55	31	10.9
5,6,7,8-Tetrahydroxycoumarin-5-β-(6-*O*-malonyl-glucopyranoside) (**2**)	malglc	OH	H	256sh; 334	256; 369	pg	t	61	41	26.0
7-Methoxy-5,6,8-trihydroxycoumarin-5-β-glucopyranoside (**3**)	glc	OH	Me	258; 310	258; 310	pg	pg	67	43	21.7
5,7,8-Trihydroxycoumarin-5-β-glucopyranoside (**4**)	glc	H	H	260; 326	281; 340; 380	pg	oy	58	37	8.0
5,7,8-Trihydroxycoumarin-5-β-(6-*O*-malonyl-glucopyranoside) (**5**)	malglc	H	H	267; 326	281; 332; 383sh	pg	oy	70	54	24.1
5,7,8-Trihydroxycoumarin-5-β-gentiobioside (**6**)	gentiob	H	H	268; 325	280; 336; 375	pg	oy	67	19	7.0
5,8-Dihydroxy-7-methoxycoumarin-5-β-glucopyranoside (**7**)	glc	H	Me	265; 319	264; 319	pg	pg	67	40	14.6
7,8-Dihydroxy-5-methoxycoumarin-7-β-sophoroside (**8**)	Me	H	soph	266; 319	266; 319	pg	pg	73	23	16.3

NA = sprayed with 0.1% diphenylboric acid β-aminoethylester in MeOH.

pg = pale green, t = turquoise green, oy = ochraceous yellow.

BAW = sec. BuOH–HOAc–H_2O (14:1:5).

HPLC = Column: 250 × 4 mm Nucleosil 100, C_{18}; 5 μm. Solvents: A = 1% aq. H_3PO_4, B = MeCN.

Elution profile: 0–8 min 8% B in A (isocratic), 8–35 min 8–25% B in A (linear gradient). 35–45 min 25% B in A (isocratic). Flow rate: 1 ml min^{-1}. Detection at 325 nm. Ambient temperature (*ca* 20°).

Table 2. ^{13}C NMR spectral data (in ppm) of glycosides **3**, **5** and **8** (DMSO-d_6, 100 MHz, ambient temp.)

Assignment	3	5	8
Aglycone			
2	159.9	160.3	160.3
3	114.0	109.2	110.7
4	140.2	139.9	140.5
5	133.0	146.7	146.6
6	135.5	100.6	96.6
7	140.1	150.8	151.2
8	133.0	127.3	128.2
9	135.9	143.5	142.7
10	110.0	103.5	104.2
OMe	60.4		56.1
Glucose			
1′	106.5	102.3	99.9
2′	73.8	73.1	82.1
3′	76.0	75.9	77.3
4′	69.6	69.8	69.6
5′	77.3	73.7	76.6
6′	60.7	63.9	60.7
1″			104.5
2″			74.5
3″			77.0
4″			69.6
5″			76.4
6″			60.7
Malonyl			
CO_2R		167.9	
CH_2		41.6	
CO_2H		166.9	

Assignments according to the literature [1, 3, 4].

Table 3. ^1H NMR spectral data (in ppm) of the coumarin glycosides **3**, **5** and **8** (DMSO-d_6, 400 MHz, ambient temp., values in parentheses: coupling constants in Hz)

H	3	5	8
Aglycone			
3	6.29 *d* (9.7)	6.09 *d* (9.6)	6.15 *d* (9.6)
4	8.29 *d* (9.7)	8.15 *d* (9.6)	8.44 *d* (9.6)
6	—	6.66 *s*	6.90 *s*
OMe	3.80 *s*	—	3.85 *s*
Sugars*			
1′	4.50 *d* (7.8)	4.69 (7.4)	4.94 *d* (7.7)
6a′	obscured	4.39 *d* (11.1)	obscured
6b′	obscured	4.10 *dd* (11.1; 6.8)	obscured
1″	—	—	4.58 *d* (7.8)

*Only signals which are clear of the multiplet representing H-2 to H-5 are presented.

An earlier report on the isolation of a dihydroflavonol from *T. pellucida* (= *Georgia pellucida*) [5] could not be substantiated during the present investigation. Since the limited data given in that report are also close to those of some of the coumarins, especially **4** and **5**, it is assumed, that in this work a mixture of coumarins was taken for a dihydroflavonol.

Thus the phenolic pattern of the *T. pellucida* gametophytes is, like that of the Polytrichaceae, characterized by tri- and tetrahydroxycoumarin derivatives. This is the first characteristic of the gametophytes that demonstrates a taxonomic relationship between Polytrichaceae and Tetraphidaceae. Anatomically the only link between the two families is provided by the sporophyte, which has in both families the peristome composed of entire cells and not, like the arthrodontous mosses, just of the walls of ruptured cells.

EXPERIMENTAL

The plant material, 280 g (dry wt) gametophytes of *Tetraphis pellucida* Hedw., was collected in December 1991 on sandstone rock west of Eppenbrunn in southern Rheinland-Pfalz, Germany. The material was identified by Prof. R. Mues and a voucher is deposited at SAAR (Nr. 3891).

Extraction and precleaning of the extract was performed as described previously [1]. Separation of the glycosides was achieved by MPLC on Lichroprep RP-18 (E. Merck, Darmstadt) with a 2% aq. HCO_2H–MeOH gradient. The glycosides were eluted between 0 and 40% MeOH, with some overlapping, in the sequence **4**, **6**, **8**, **1**, **3**, **2**, **5**, **7**. Final cleaning was performed by CC on Sephadex LH 20 with H_2O or H_2O–MeOH as eluents. By these methods we isolated in the pure state: 39 mg **1**, 4 mg **2**, 9 mg **3**, 18 mg **4**, 3 mg **5**, 12 mg **6**, 5 mg **7** and 4 mg **8**.

FAB-MS: Finnigan MAT 90 with 5–8 keV Xe and glycerol as matrix; NMR and UV: Tables 1–3.

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