The First Biflavone Found in Liverworts and Other Phenolics and Terpenoids from *Chandonanthus hirtellus ssp. giganteus* and *Plagiochila asplenioides*

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A biflavone was isolated from a liverwort (*Chandonanthus hirtellus ssp. giganteus*) for the first time. Its structure was determined as dicranolomin. Moreover luteolin and the new compound vanillic acid-4-O-neohesperidoside were identified from this *Chandonanthus* species. From *Plagiochila asplenioides* we isolated the new sesquiterpenoid (-)-gymnomitr-8(12)-en-9β-ol with chair conformation of the cyclohexane ring together with plagiochiline P and the diterpenoid anadensin, both new to this liverwort.

Introduction

The leafy liverwort genus *Chandonanthus* belongs to the family Lophoziaceae. After the separation of *Tetralophozia* (Schust.) Schljakov the genus is composed of less than 10 species (Schuster, 1983), according to Schuster (1969) "chiefly antipodal in range". Although the chemistry of Lophoziaceae is comparatively well known (Asakawa, 1995), secondary products have so far been isolated from only one *Chandonanthus*-species, *C. hirtellus ssp. hirtellus*, widely distributed in paleotropic regions (Vána, 1993). This species produces various *n*-alkanes, sesquiterpenes and the cembrane-type diterpene chandonanthone (Asakawa, 1995; Asakawa *et al.*, 1991; Matsuo *et al.*, 1986). *Chandonanthus hirtellus ssp. giganteus* only occurs in central African mountains (Vanden Berghen, 1965; Vána, 1993). 2D-TLC-surveys of its methanolic extract prompted us to undertake a detailed phytochemical investigation for chemotaxonomic reasons.

In contrast, the liverwort *Plagiochila asplenioides* was the object of several phytochemical studies since more than 20 years yielding a wide array of secondary products (Asakawa, 1995). In the course of a further investigation in our laboratory we isolated three minor compounds, so far not reported from this species.

Results and Discussion

Chandonanthus hirtellus ssp. giganteus

Chromatographic separation of the 80% aq. MeOH extract yielded three phenolic compounds in sufficient amounts to allow structure elucidation. Apart from luteolin (**1**) we found another flavonoid compound (**2**). The analysis of its spectroscopic data (see *Experimental*) confirmed that **2** was identical with dicranolomin (2',6''-biluteolin), a biflavone first isolated from the moss *Dicranoloma robustum* and so far being unique for mosses (Markham *et al.*, 1988; Geiger *et al.*, 1997). This is the first report of a biflavone from a liverwort and quite surprising because among bryophytes biflavonoids have so far been considered to be chemotaxonomic markers for mosses only. An account on the occurrence of moss biflavonoids was recently published (Geiger *et al.*, 1997).

The more polar compound **3** afforded NMR-spectroscopic data (Table I) typical for a glycosylated phenolic acid with a 1,3,4-trisubstitution of the aromatic ring according to the shift and coupling pattern of H-2, H-5, and H-6 respectively. In addition, a methoxyl group was visible at 3.75 ppm. A carboxylic function could be derived from the signal at 168.9 ppm in the ^{13}C NMR

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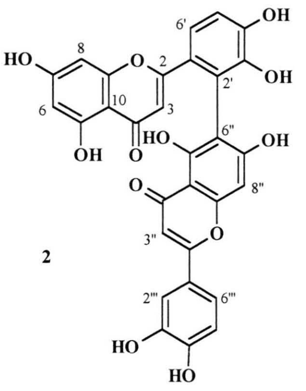
Table I. ^1H (400 MHz) and ^{13}C (100 MHz) NMR data of **3** (DMSO-d_6).

H	3		C	3
–			1	130.0
2	7.47 *brs*		2	112.6
–			3	148.1
–			4	147.9
5	7.01 *d*	(8.4)	5	113.8
6	7.41 *brd*	(8.2)	6	121.7
–			7	168.9
OMe-3	3.75 *s*		OMe-3	55.3
1'	5.07 *d*	(7.3)	1'	97.8
2'	3.54 *m*		2'	75.5
3'	3.50 *m*		3'	77.8
4'	3.22 *m*		4'	69.9
5'	3.35 *m*		5'	76.8
6'a	3.65 *m*		6'	60.5
6'b	3.45 *m*			
1"	5.25 *brs*		1"	99.8
2"	3.68 *m*		2"	70.6
3"	3.35 *m*		3"	70.4
4"	3.18 *m*		4"	71.9
5"	3.90 *m*		5"	68.2
Me-6"	1.11 *d*	(6.0)	6"	18.0



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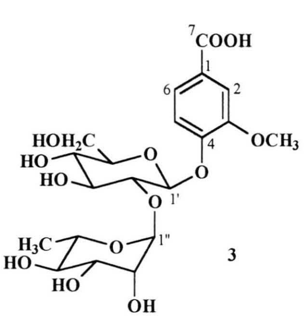

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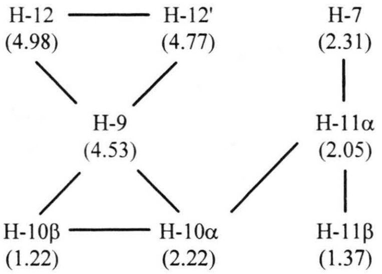
spectrum. Further analysis of the ^1H and ^{13}C NMR data resulted in the identification of a β-D-glucose and a α-L-rhamnose moiety. They were linked *via* 1–2 interglycosyl linkage between rhamnose and glucose (i.e. neohesperidoside) because of the downfield shift (> 2 ppm) of C-2 of the glucosyl part compared to a monoglucoside (Markham and Chari, 1982). Moreover a heteronuclear long-range correlation existed between H-1 of the rhamnose (5.25 ppm) and C-2 of the glucose (75.5 ppm). Glucose-H-1 (5.07 ppm) exhibited a long-range coupling to the carbon at 147.9 ppm, which was assigned to C-4 of the aromatic ring. With the help of the 2D NMR spectra (H-H, C-H, long-range C-H COSY, NOESY) it was possible to assign all signals and to identify **3** as vanillic acid-4-O-neohesperidoside, apparently a new natural product. The FAB-MS with $[M]^-$ at *m/z* 476 ($C_{20}H_{28}O_{13}$) also coincided with the structure. Several other benzoic acid-derivatives have already been reported for bryophytes (Asakawa, 1995).

Plagiochila asplenioides

The lipophilic extract yielded, apart from known compounds (Asakawa, 1995), the seco-aromadendrane-type sesquiterpenoid plagiochiline P (**4**) (Valcic *et al.*, 1997) and the diterpenoid anadensin (**5**) (Huneck *et al.*, 1983). Their spectroscopical data were in agreement with published ones. These two terpenoids are new to *P. asplenioides.*

In addition we found another sesquiterpenoid (**6**) with $[M+H]^+$ at *m/z* 221 ($C_{15}H_{24}O$). Together with the NMR spectra we assumed a tricyclic molecule with an exomethylene (δ_H 4.98 and 4.77 ppm; δ_C 107 ppm), a hydroxyl (δ_H 4.53 ppm; δ_C 68.3 ppm) and three methyl groups (δ_H 1.02, 0.88 and 0.86 ppm; δ_C 27.7, 24.1 and 23.2 ppm). The analysis of the H-H COSY spectrum revealed two sets of sequences. The first sequence comprised the protons of the exomethylene group (H_2-12), the oxygenated methine protone (H-9; allylic coupling to H_2-12 not visible in the ^1H NMR spectrum), a methylene group (H_2-10, vicinal to H-9), another methylene group (H_2-11; not vicinal to H_2-10; only ^4J coupling between H-11α and H-10α), and finally an allylic (δ 2.32 ppm) methine proton (H-7) which was vicinal to H_2-11 (Scheme 1; for coupling constants see Table II).



Scheme 1. ^{1}H–^{1}H correlations of **6** detected by COSY (chemical shifts in parentheses).

Table II. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **6** ($CDCl_3$).

H	6		C	6
–	–		1	54.6
–	–		2	45.4
3α	1.85 *m*		3	35.9
3β	1.15 *m*			
4α	1.82 *m*		4	27.3
4β	1.75 *m*			
5α	1.70 *m*		5	37.4
5β	1.19 *m*			
–	–		6	55.7
7	2.31 *d*	(4.7)	7	56.5
–	–		8	154.4
9	4.53 *dd*	(8.0/10.0)	9	68.3
10α	2.22 *ddd*	(3.0/7.8/13.4)	10	49.8
10β	1.22 *m*			
11α	2.05 *ddd*	(3.1/4.9/11.5)	11	47.0
11β	1.37 *d*	(11.5)		
12	4.98 *brs*		12	107.0
12'	4.77 *brs*			
Me-13	0.86 *s*		13	24.1
Me-14	0.88 *s*		14	23.2
Me-15	1.02 *s*		15	27.7

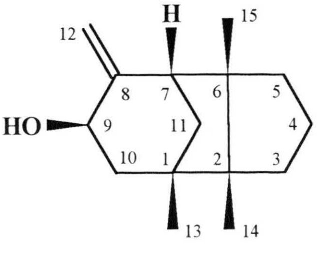
The second sequence only consisted of the remaining methylenes (H_2-3, H_2-4, H_2-5) which were coupled only among themselves. Using further 2D NMR spectra (C-H and long-range C-H COSY) we were able to assign all signals (Table II) and to identify **6** as gymnomitr-8(12)-en-9-ol. The gymnomitrane skeleton is unique for liverworts. So far (+)-gymnomitr-8(12)-9α-ol (**7**) was reported from the liverwort *Reboulia hemisphaerica* (Morais *et al.*, 1988; Morais, 1990). However, **6** exhibited quite different NMR data than **7**. Additionally **6** was characterized by a negative optical rotation value. Thus **6** and **7** should be different in their stereochemistry. Irradiation experiments (NOESY) of **6** showed that saturation of H-9 (δ 4.53 ppm) enhanced the signals of two protons (δ 1.85 and 1.70 ppm) belonging to the methylene group at C-3 and C-5, respectively, which were assigned to be H-3α and H-5α. Additionally, a weak enhancement of the proton at δ 2.22 ppm (H-10α) was also observed. Studying the respective NOE results of **7** (Morais *et al.*, 1988; Morais, 1990), irradiation of H-9 raised the resonance of H-11β and H-7. These NMR experiments proved that the cyclohexane ring of **6** was in the chair form, whereas the boat form was present in **7** (Fig. 1). Thus **6** could be elucidated as (-)-gymnomitr-8(12)-en-9β-ol (relative stereochemistry), a diastereomer of **7**.

Experimental

Plant material

Chandonanthus hirtellus (Web.) Mitt. *ssp. giganteus* (Steph.) Vanden Berghen was collected in September 1991 in Zaire, on Mt. Kahuzi, altitude 3200 m, Kahuzi-Biega National Park. It was identified by Prof. Dr J.-P. Frahm, Bonn. Voucher specimens are deposited in the herbarium SAAR, no. 5170, Saarbrücken.

Plagiochila asplenioides was collected in November 1995 on rocks in a forest (altitude ca. 450 m) near Birresborn (Eifel, Rhineland-Palatinate), Germany. Voucher specimens are deposited in the herbarium SAAR, no. 5457, Saarbrücken.



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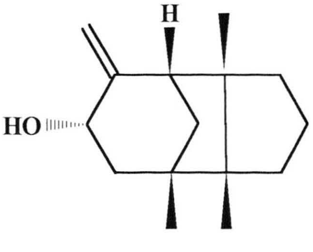

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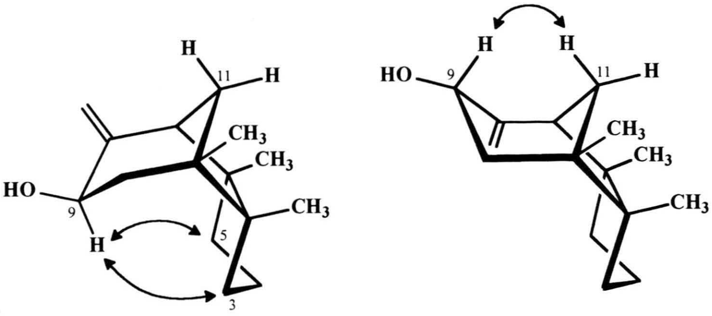

6 **7**

Fig. 1. Stereochemical view of **6** and **7** with significant NOEs.

Extraction and isolation
Chandonanthus hirtellus ssp. giganteus

140 g of cleaned, air-dried gametophytic material were ground in a blendor and preextracted x4 with 1200 ml CH_2Cl_2 to remove lipophilic compounds, followed by extraction 6 times with 1200 ml 80% aq. MeOH. This crude extract was prefractionated by vacuum liquid chromatography (VLC) on RP 18 (H_2O-MeOH-gradient) to yield two major fractions (fr. 1: eluted with 20–40% aq. MeOH; fr. 2: eluted with 70–90% aq. MeOH). Further chromatographic steps of fr. 1 including prep. HPLC (solvent: H_2O-MeOH-mixts.) and final column chromatography (CC) on Sephadex LH-20 (solvent: 50% aq. MeOH) were necessary to yield pure **3** (30 mg). Fr. 2 was chromatographed on Sephadex LH-20 (solvent: Me_2CO-MeOH-H_2O 2:1:1, v/v/v) to separate **1** from **2**. Final purification of **1** was achieved by prep. HPLC (solvent: 55% aq. MeOH) to yield 3 mg of **1**. Additional CC on Sephadex LH-20 (solvent: 90% aq. MeOH) resulted in 4 mg of **2**.

Plagiochila asplenioides

750 g of cleaned, air-dried gametophytic plant material were ground in a blendor and extracted 8 times with 5000 ml CH_2Cl_2. The terpenoids were isolated using standard procedures like CC on Sephadex LH-20, VLC on silica gel and RP 18, and prep. HPLC on silica gel, diol, and RP 18 respectively as mentioned in Kraut *et al.* (1997). Yields: **4**: 12 mg; **5**: 11 mg; **6**: 17 mg.

Chromatography

Adsorbents for TLC: Schleicher & Schüll cellulose, Avicel, F 1440; Macherey & Nagel Polygram polyamide-6; Merck RP 18 F_{254s}; Merck silica gel 60 F_{254}; Merck diol F_{254s}; solvents for TLC: *tert.* BuOH-HOAc-H_2O 3:1:1 and 15% HOAc (cellulose); EtOAc-EtCOMe-HOAc-H_2O 5:3:1:1, v/v/v/v and Me_2CO-HOAc-H_2O 3:1:1, v/v/v (polyamide); mixtures of MeOH with 5% HOAc (RP 18); mixtures of *n*-hexane with EtOAc (silica gel, diol). HPLC: LiChrospher 100 RP 18, 5 μm, Merck, 250 x 4 mm; LiChrospher 100 RP 18, 10 μm, Merck, filled by Muder & Wochele Comp., Berlin, 250 x 16 mm; LiChrospher 100 diol, 5 μm, Merck, 250 x 4 mm; Lichrospher 100 diol, 10 μm, Merk, 250 x 10 mm; Nucleosil 100 Si, 5 μm, Macherey & Nagel, 250 x 4 mm; Nucleosil 100 Si, 7 μm, Macherey & Nagel, 250 x 10 mm. Detection on TLC-plates: UV-light, spray reagents (Naturstoffreagenz A for flavonoids; anisaldehyde-H_2SO_4 for terpenoids on silica gel).

Spectroscopic data

NMR: 400 MHz (1D) and 500 MHz (2D; HETCOR: inverse technique). Mass spectra were recorded using FAB-MS (4–6 keV, Xe, glycerol as matrix, negative mode) and EI-MS (70 eV). The optical rotation value of **6** was determined at 25 °C at 589 nm in $CHCl_3$ (Uvasol).

Compound **2**. UV: λ_{max}, MeOH: 346–254. ^1H NMR: δ (ppm; DMSO-d_6): 13.15 (1H, *s*, H-5"), 12.80 (1H, *s*, H-5), 7.42 (1H, *dd*, J = 2.4/8.2 Hz, H-6"'), 7.40 (1H, *d*, J = 2.2 Hz, H-2"'), 7.16 (1H, *d*, J = 8.4 Hz, H-5"'), 6.91 (1H, *d*, J = 8.4 Hz, H-6'), 6.89 (1H, *d*, J = 8.8 Hz, H-5'), 6.65 (1H, *s*, H-3"), 6.49 (1H, *s*, H-8"), 6.08 (1H, *d*, J = 1.8 Hz, H-8), 6.03 (1H, *s*, H-3), 5.97 (1H, *d*, J = 1.8 Hz, H-6). ^{13}C NMR: δ (ppm; DMSO-d_6): 181.5, 181.3, 166.6, 163.9, 161.3, 158.7, 157.4, 156.4, 149.6, 148.5, 145.7, 144.6, 123.8, 121.6, 120.1, 118.9, 118.3, 116.1, 114.0, 113.3, 108.3, 106.1, 103.4, 102.8, 98.6, 93.5, 93.3. For comparison see Markham *et al.* (1988) and Seeger *et al.* (1992). FAB-MS m/z 570 $[M]^-$.

Compound **3**. UV: λ_{max} (MeOH): 291–253. NMR data: see Table I. FAB-MS: m/z 476 $[M]^-$.

Compound **6**. NMR data: see Table II. EI-MS: m/z (rel. intensities,%): 221 $[M+H]^+$ (2.7), 187 (2.4), 123 (100), 96 (99), 81 (98). $[\alpha]_D$ −33.8° (c 0.34).

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