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PHYTOCHEMISTRY

Lignans from the liverworts *Lepidozia incurvata*, *Chiloscyphus polyanthos* and *Jungermannia exsertifolia* ssp. *cordifolia*[☆]

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Abstract

Three foliose liverworts from the order Jungermanniales were examined for the presence of lignans. They all contained the typical liverwort lignans, i.e. cyclolignans of the dihydronaphthalene and naphthalene type and conjugated with α-L-rhamnose, malic acid and shikimic acid, respectively. The new lignans were 3-carboxy-6-methoxy-1-(3′,4′-dihydroxyphenyl)-naphthalene-7-*O*-α-L-rhamnopyranoside, 3-carboxy-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl)-naphthalene-9,2″-*O*-malic acid ester and 3-carboxy-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl)-naphthalene-9,5″-*O*-shikimic acid ester.

Keywords: liverworts; *Lepidozia incurvata*; *Chiloscyphus polyanthos*; *Jungermannia exsertifolia ssp. cordifolia*; Jungermanniales; lignans; α-L-rhamnose; Malic acid; Shikimic acid

1. Introduction

Whilst the phytochemical investigation of the lipophilic constituents and the flavonoids of liverworts had already resulted in a broader knowledge of the natural products of this long neglected group of plants (Asakawa, 1995), only a few reports deal with another group of their secondary metabolites, the lignans (Cullmann, Adam & Becker, 1993; Cullmann, Adam, Zapp & Becker, 1996; Martini, Zapp & Becker, 1998; Mues, Huneck, Conolly & Rycroft, 1988; Rischmann, Mues, Geiger, Laas & Eicher, 1989; Tazaki, Adam & Becker, 1995). Continuing our study of the presence and structural variation of lignans in liverworts, we examined the three foliose liverworts, *Lepidozia incurvata*, *Chiloscyphus polyanthos* and *Jungermannia exsertifolia* ssp. *cordifolia*, all belonging to the order Jungermanniales.

Lepidozia incurvata, Lepidoziaceae, was collected in Costa Rica and had not been phytochemically investigated so far. *Chiloscyphus polyanthos*, Geocalycaceae, collected in Germany, is one of the three species of this genus in the Northern hemisphere (Müller, 1954). There are only reports on the lipophilic constituent, e.g. GC-MS analysis (Asakawa, Matsuda, Toyota, Takemoto, Connolly & Phillips, 1983; Asakawa, Toyota, Takemoto & Suire, 1979) and a phytochemical examination of Japanese *Ch. polyanthos* resulted in two sesquiterpenes with new skeletons, chiloscyphone and chiloscypholone (Tori, Hasebe & Asakawa, 1990; Tori, Takeshi & Asakawa, 1988). From *Jungermannia exsertifolia* ssp. *cordifolia*, Jungermanniaceae, obtained from Switzerland, no examination of hydrophilic compounds is reported either. However, there were reports on the lipophilic constituents of this species from France, yielding highly oxygenated kaurane-type diterpenoids (Nagashima, Tanaka, Takaoka & Asakawa,

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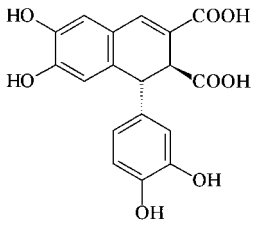
1996), of a Walsh *J. exsertifolia* (Harrison & Asakawa, 1989) and on sterols (Huneck, Jänicke & Schmidt, 1984).

2. Results and discussion

In all three cases, the lignans were isolated from the ethyl acetate soluble part of the methanolic extract obtained after removing the lipophilic constituents by extraction with diethyl ether. The typical isolation scheme comprised CC on Sephadex LH20, VLC on diol modified silicagel or reversed phases and preparative HPLC on these supports.

Thus, *L. incurvata* yielded the typical lignans 2,3-dicarboxy-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl)-1,2-dihydronaphthalene (**1**) and its derivative 3-carboxy-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl)-naphthalene



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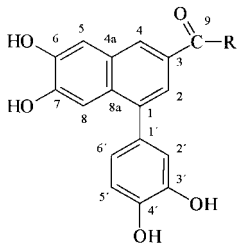

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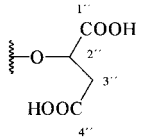

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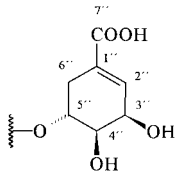

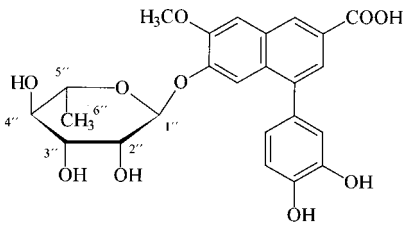

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Table 1
^{1}H NMR spectral data and coupling constants (in Hz in parentheses) of compounds **3**, **4** and **5** (MeOH-*d4*)

H	3	4	5
Lignan moiety			
H-2	7.64 d (1.7)	7.62 d (1.7)	7.75 brs
H-4	8.28 d (1.7)	8.25 d (1.7)	8.46 brs
H-5	7.27 s	7.26 s	7.46 s
H-8	6.26 s	7.26 s	7.67 s
H-2′	6.88 d (2.1)	6.87 d (2.2)	6.88 d (1.8)
H-5′	6.89 d (7.9)	6.90 d (8.0)	6.88 d (8.1)
H-6′	6.77 dd (7.9, 2.1)	6.76 dd (8.0, 2.2)	6.75 dd (8.1, 1.8)
O-Methyl	–	–	3.98 s
Conjugate	Malic acid	Shikimic acid	α-L-rhamnose
H-1″	–	–	5.33 brs
H-2″	5.63 dd (7.0, 4.6)	6.87 s	4.06 m
H-3″	3.03 m	4.47 brs	3.87 m
H-4″	–	4.03 dd (7.1, 4.1)	3.43 t (9.5)
H-5″	–	5.41 dd (11.9, 5.1)	3.65 m
H-6″α	–	2.94 dd (18.4, 5.1)	1.19 d (6.2)
H-6″β	–	2.42 dd (18.4, 5.1)	

(**2**), first isolated from *Pellia epiphylla* (Cullmann et al., 1993; Rischmann et al., 1989) and later found in *Jamesoniella autumnalis* (Tazaki et al., 1995), *Bazzania trilobata* (Martini et al., 1998) and *Lepidozia reptans* (Sanders, 1996).

Table 2
: ^{13}C NMR spectral data of compounds **3**, **4** and **5** (MeOH-*d4*)

Carbon	3	4	5
Lignan moiety			
C-1	140.2 s	140.2 s	140.5 s
C-2	124.3 d	122.3 d	124.9 d
C-3	124.7 s	125.3 s	126.5 s
C-4	129.5 d	129.1 d	129.3 d
C-4a	130.3 s	130.3 s	130.8 s
C-5	112.3 d	112.2 d	109.4 d
C-6	148.3 s	148.2 s	151.4 s
C-7	150.3 s	150.1 s	148.4 s
C-8	109.5 d	109.4 d	113.1 d
C-8a	132.1 s	131.9 s	130.6 s
C-9	167.9 s	168.3 s	167.7 s
C-1′	133.8 s	133.8 s	132.4 s
C-2′	118.0 d	118.0 d	117.8 d
C-3′	145.9 s	145.9 s	145.6 s
C-4′	146.2 s	146.1 s	145.6 s
C-5′	116.3 d	116.3 d	116.1 d
C-6′	122.4 d	122.4 d	122.1 d
O-Methyl	–	–	55.6 q
Conjugate	Malic acid	Shikimic acid	α-L-rhamnose
C-1″	173.0[a] s	130.3 s	100.8 d
C-2″	70.5 d	138.9 d	71.8 d
C-3″	37.2 t	67.4 d	72.1 d
C-4″	172.6[a] s	70.1 d	73.6 d
C-5″	–	71.9 d	70.8 d
C-6″	–	29.2 t	17.8 q
C-7″	–	169.6 s	

[a] Assignments may be interchanged in vertical columns.

The third compound (**5**) showed in its ^{1}H NMR spectra the existence of the phenylnapthalene lignan **2** as a partial structure. Additional signals were a methoxy group (δ_H 3.98, 3 H, s) and a sugar moiety (δ_H 5.33, 1 H; δ_H 4.06–3.43, 4 H, δ_H 1.19, 1 H). Since the amount of the substance was too small to obtain a ^{13}C NMR spectrum, these data were taken from the ^{13}C projection of the HSQC and HMBC spectra, in which all ^{13}C signals were present. Due to its proton and carbon NMR data (Table 1 and 2), especially the doublet methyl signal at δ_H 1.19 (6.2 Hz), this sugar moiety could be identified unambiguously as an α-rhamnopyranose. The connections of these three subunits were achieved by the HMBC spectrum. There was a correlation visible between H-1″ (δ_H 5.33) of the rhamnose moiety and the carbon C-7 (δ_C 148.4) of the lignan moiety. The same holds true for the methyl signal (δ_H 3.88) and C-6 (δ_C 151.4), explaining the differences in the ^{13}C NMR data of C-5, C-6, C-7 and C-8 of compound **5** compared to the free naphthalene lignan **2**. Thus, compound **5** is the new 3-carboxy-6-methoxy-1-(3′,4′-dihydroxyphenyl)-naphthalene-7-*O*-α-L-rhamnopyranoside.

From *Ch. polyanthos*, again, compounds **1** and **2** were obtained along with another derivative of **2**. This compound, **3**, had four additional signals in the ^{13}C NMR spectrum: two carboxylic carbon atoms (δ_C 173.0, 172.6), one oxygen bearing methine group (δ_C 70.5) and a methylene group (δ_C 37.2), which could be assigned as the signals of malic acid. Due to the high-field shift of H-2″ (δ_H 5.63) of the malic acid moiety, an ester bond between the alcoholic function of the malic acid and the carboxylic atom of the lignan was assumed. This assumption was proved by a correlation between that alcoholic proton and the carboxylic carbon atom in the HMBC spectrum, giving the structure of the new 3-carboxy-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl)-naphthalene-9,2″-*O*-malic acid ester (**3**).

The extract of *J. exsertifolia* ssp. *cordifolia* did not contain compound **1**, but **2** was present in a high amount together with compound **4**, in which this lignan was conjugated with a shikimic acid moiety. A similar compound, where the shikimic acid moiety was esterified via the alcoholic function of C-5″ to the lignan **1** had already been found in *Pellia epiphylla* (Cullmann et al., 1993) and *Lepidozia reptans* (Sanders, 1996). As the data of the shikimic acid moiety of compound **4** were identical to those from *Pellia epiphylla* and the HMBC spectrum showed the expected correlation between H-5″ (δ_H 5.41) and C-9 (δ_C 168.3), compound **4** is the new 3-carboxy-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl)-naphthalene-9,5″-*O*-shikimic acid ester.

Once more, liverworts proved to be a source for new lignans. In this case, however, these three liverworts produce rather simple lignan derivatives compared with the structures of pelliatin from *Pellia epiphylla* (Cullmann et al., 1996) or the lignan derivatives isolated from *Bazzania trilobata* (Martini et al., 1998).

3. Experimental

Solvents used for spectral measurements: MeOH-d_4 (^{1}H NMR: 400 MHz; ^{13}C NMR: 100 MHz for 1D, 500 MHz and 125 MHz for 2D techniques, respectively. Chemical shifts are given in δ values from TMS), MeOH (optical rotation).

3.1. Plant material

Lepidozia incurvata Lindenberg (700 g) was collected in September 1994 near volcano Poás, Costa Rica and identified by Mues and Gradstein.

Chiloscyphus polyanthos (L.) Corda (960 g) was collected in June 1993 in Hoof, Saarland, Germany by F. C. and F. S. and identified by Mues.

Jungermannia exsertifolia Steph. ssp. *cordifolia* (Dum.) Vana (390 g) was collected in October 1995 near Oberalppaß, Swiss Alps, by M. L. T. and identified by Mues. All voucher specimen are deposited in the Herbarium of the Fachrichtung 12.3 Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes.

3.2. Extraction and isolation

The extraction scheme followed the standard procedure of our group (Cullmann et al., 1993; Martini et al., 1998).

3.3. Lepidozia incurvata

The ethyl acetate soluble part of the methanolic extract was sep. by CC on Sephadex LH20 (MeOH/CH_2Cl_2 80/20) to yield two lignan containing frs. The first fr. was subjected to a VLC on diol modified silica gel in a hexane–ethyl acetate gradient and gave 10 mg of **2**. The second fr. was prepurified by VLC on RP18 in a water–methanol gradient giving rise to two subfrs., whose purification by HPLC on RP18 yielded 2 mg of **1** (H_2O/MeOH 75/25 + 1% HCOOH) and 2 mg of **5** (H_2O/MeOH 80/20 + 1% HCOOH).

3.4. Chiloscyphus polyanthos

From the CC on Sephadex LH20 (MeOH/CH_2Cl_2 80/20) two main frs. were obtained. VLC of the first fr. on diol modified silica gel in a hexane–ethyl acetate gradient gave two subfrs. Preparative HPLC on cyano phases of these subfrs. gave 5.7 mg of **3** (hexane/EtOAc 65/35) and 14.9 mg of **2** (hexane/EtOAc 55/45).

The second main fr. was prepurified by a VLC (analogous to the one above) and the final isolation succeeded by HPLC on RP18 (H_2O/MeOH 70/30) to yield 40.5 mg of **2**.

3.5. *J. exsertifolia ssp. cordifolia*

Again, CC on Sephadex LH20 directly gave 240 mg of **2** and a fr., whose purification by HPLC on diol modified silicagel (MeOH/EtOAc 4/96) yielded 22.7 mg of **4**.

3.6. *Compound* **3**

$[\alpha]_D^{20} = -7.5^\circ$ (*c* 0.28). ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. FABMS: $[M+H]^+$ *m/z* 429.

3.7. *Compound* **4**

$[\alpha]_D^{20} = -14.0^\circ$ (*c* 0.88). ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. EIMS: $[M]^+$ *m/z* 468.

3.8. *Compound* **5**

$[\alpha]_D^{20} = -72.0^\circ$ (*c* 0.18). ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. FABMS: $[M+H]^+$ *m/z* 473.

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