PERGAMON

Phytochemistry 52 (1999) 1651–1656

PHYTOCHEMISTRY

Lignans from the liverwort *Lepicolea ochroleuca*[☆]

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Received 26 April 1999; received in revised form 23 June 1999; accepted 25 June 1999

Abstract

The polar extract of *Lepicolea ochroleuca* (Lepicoleaceae) yielded four new lignan conjugates of the most common lignan from liverworts, 2,3-dicarboxy-6,7-dihydroxy-1-(3′,4′-dihydroxy)-phenyl-1,2-dihydronaphthalene, for which the name epiphyllic acid is introduced. Additional compounds were the two already known 2,4,6-trihydroxyacetophenone-3,5-di-C-glucoside and protocatechuic acid-4-*O*-β-glucoside. By means of modern spectroscopic methods, the new lignans were assigned as epiphyllic acid-7-*O*-β-glucoside-10-methyl ester, epiphyllic acid-7-*O*-β-glucoside-10,5‴-*O*-shikimic acid ester, epiphyllic acid-7-*O*-β-glucoside-9,1⁗-*O*-heptitol ester-10,5‴-*O*-shikimic acid ester, and epiphyllic acid-9,5‴-*O*-,10,5⁗-*O*-bis(shikimic acid ester). The chemosystematic of the lignans from liverworts is discussed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Lepicolea ochroleuca; Liverwort; Cyclolignans; Lignan conjugates; Shikimic acid; Epiphyllic acid; Acetophenone-di-C-glucoside; Protocatechuic acid glucoside

1. Introduction

Liverworts are a large and yet both biologically and chemically uncompletely examined class of bryophytes. Due to their morpholocical variation, the small degree of different tissues and organs and the lack of flowering parts, chemosystematic findings are a useful tool for systematic studies. This advantage had already been shown in the case of terpenoid constituents (Asakawa, 1995). But in the last years, another group of natural compounds, the lignans, were found to be present in liverworts (Cullmann, Adam, Zapp & Becker, 1996; Cullmann, Schmidt, Schuld, Trennheuser & Becker, 1999; Martini, Zapp & Becker, 1998; Tazaki, Adam & Becker, 1995). Here, even more limited knowledge is present and a great effort is necessary to obtain sufficient information for the still puzzling findings.

In this course of our study for lignans from liverworts, we examined the methanolic extract of the liverwort *Lepicolea ochroleuca* from Chile. It is a large (to 14 cm), foliose liverwort with rigid stems growing in large, deep cushions. It belongs to the Gondwanaland flora with occurrences in South Africa, New Zealand and especially South America, reaching up to Mexico. So far, besides one GC-MS examination of *Lepicolea pruinosa* (Gradstein, Matsuda & Asakawa, 1981), no phytochemical examination has been published for the genus *Lepicolea.*

2. Results and discussion

The butanol soluble part of the methanolic extract of *L. ochroleuca* was examined and yielded the unusual 2,4,6-trihydroxyacetophenone-3,5-di-C-glucoside **5**, already known from *Melicope pteleifolia* (Chen, Lin, Chou & Chen, 1994) and the liverwort *Scapania nemorosa* (Geis, 1999), the protocatechuic acid-4-*O*-β-glucoside (**6**), described from the fern *Angiopteris lygodiifolia* (Murakami & Tanaka, 1988) and *Picea glauca* (Kraus & Spiteller, 1997), and four lignan conjugates

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[☆] Publication No. 140 in the series of ‘Arbeitskreis Chemie und Biologie der Moose’. For part 139, see Flegel and Becker (1999).

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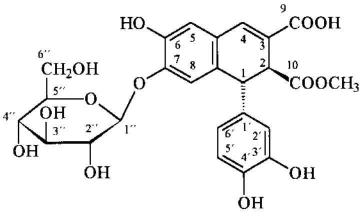
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00372-6

1–**4**, which were derivatives of the most common lignan from liverworts, 2,3-dicarboxy-6,7-dihydroxy-1-(3′,4′-dihydroxy)-phenyl-1,2-dihydronaphthalene.

When we first isolated this lignan from *Pellia epiphylla* (Cullmann, Adam & Becker, 1993), we used the systematic name for this compound, but since then, a lot of derivatives of this moiety were isolated and the names of these compounds became unduly long and complicated (Martini et al., 1998; Sanders, 1996). Therefore, we want to introduce a trivial name for this cyclolignan: epiphyllic acid, after the first isolation from *P. epiphylla.*



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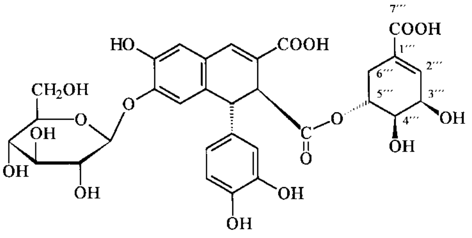

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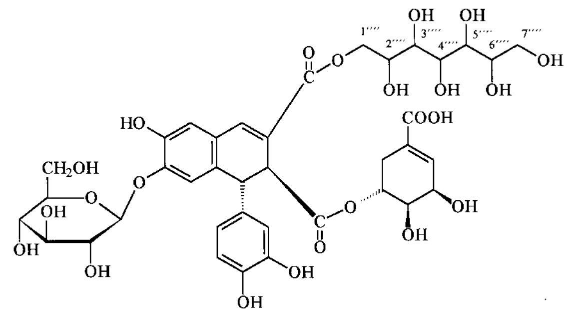

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Compound **1** showed in its ^{1}H and ^{13}C NMR data the typical signals of the cyclolignan moiety epiphyllic acid (see Tables 1 and 2). Additionally, there were the signals of a methyl ester (δ_H 3.58, *s*; δ_C 52.7, *q*) and six carbon atoms with the typical shifts of a sugar moiety. This sugar moiety was recognised as β-glucose due to the ^{13}C NMR shifts and the coupling constant of proton H-1 (J = 7.1 Hz). To determine the position of these moieties, a HMBC spectrum was measured. The methyl ester showed a correlation to the carboxylic acid C-10 (δ_C 175.2) of the epiphyllic acid; the anomeric proton of the glucose moiety showed a correlation to C-7 of that lignan. The discrimination of the two carboxylic acid moieties is rather simple due to their large difference in the chemical shift of the ^{13}C carbon atoms. Since the determination of C-6 and C-7 is not unequivocal, due to the difficulties in discriminating the ^{2}J and ^{3}J correlations of H-5 and H-8 together with the narrowness of these signals in the ^{1}H NMR spectrum, a NOESY spectrum was run and gave the final proof. Here, the proton H-5 can easily be distinguished from H-8 because of the correlations between H-4 and H-5. Similarly, the proton H-8 can be determined by a correlation between H-1 and H-8. As the anomeric proton of the sugar showed a correlation to H-8, the assignment above holds true. Thus, compound **1** is the new epiphyllic acid-7-*O*-β-glucoside-10-methyl ester. However, we have to discuss the possibility of being an artefact by trans-esterification during extraction with methanol.

Compound **2** showed close similarities to compound **1** in its NMR spectra, the differences being the lack of the methyl ester and the appearance of signals for a third partial structure. This moiety was recognised as 5-*O*-shikimic acid ester, already known as partial structure of other liverwort lignans (Cullmann et al., 1993; 1999; Sanders, 1996). Unlike these previously reported lignans, the HMBC correlations showed the shikimic acid to be connected to C-10. Again, the structure of the remaining partial structures and the position of the glucose moiety was confirmed by HMBC and NOESY experiments, giving the structure of epiphyllic acid-7-*O*-β-glucoside-10,5‴-*O*-shikimic acid ester.

Probably, compound **1** arose from **2**, because from *P. epiphylla* both epiphyllic acid-9,5″-*O* shikimic acid ester and epiphyllic acid-9-methyl ester were isolated (Cullmann et al., 1993).

Compound **3** was the most complex structure from *L. ochroleuca*. Again, the highfield shift area of the ^{1}H NMR spectrum contained the signals of epiphyllic acid. The area between δ_H 4.8–3.3, however, was characterised by a lot of overlapping signals, preventing any structure elucidation. In the ^{13}C NMR spectrum, 38 resolved signals were present, allowing to attribute 31 signals to the partial structure of compound **2**. The remaining seven signals were two oxygen bearing methylene groups (δ_C 67.4, 64.1) and five oxygen bearing methine groups (δ_C 74.9, 74.1, 73.2, 72.3, 71.1).

Despite the difficulties due to the overlapped and unresolved spin systems, the H–H-COSY and HMBC spectra revealed the existence of a heptitol, an unbranched chain of seven oxygen bearing carbon atoms. The highfield shift of H-1‴ (δ_H 4.40, 4.28, *dd* each) indicated an ester bond between this centre and

Table 1
^{1}H NMR spectral data and coupling constants (in Hz in parentheses) for compounds **1**, **2**, and **3** (Methanol-d_4) (nr; not resolved)

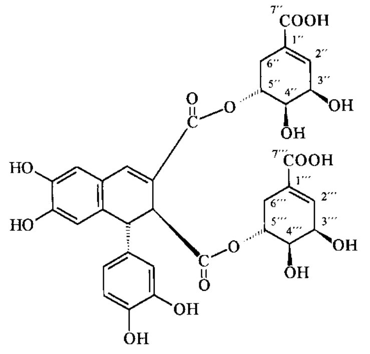
H	**1**	**2**	**3**
Lignan moiety			
1	4.41 *d* (4.4)	4.34 *d* (4.9)	4.35 *d* (4.5)
2	3.90 *d* (4.4)	3.88 *d* (4.9)	3.95 *d* (4.5)
4	7.59 *s*	7.57 *s*	7.70 *s*
5	6.89 *s*	6.87 *s*	6.94 *s*
8	6.90 *s*	6.85 *s*	6.82 *s*
2′	6.49 *d* (2.1)	6.49 *d* (2.1)	6.54 *d* (1.8)
5′	6.63 *d* (8.2)	6.64 *d* (8.2)	6.68 *d* (8.2)
6′	6.41 *dd* (8.2, 2.1)	6.41 *dd* (8.2, 2.1)	6.41 *dd* (8.2, 1.8)
Glucose moiety			
1″	4.76 *d* (7.1)	4.79 *d* (7.5)	4.84 *d* (7.3)
2″	3.45 nr	3.46 nr	3.52 nr
3″	3.44 nr	3.46 nr	3.52 nr
4″	3.42 nr	3.42 nr	3.48 nr
5″	3.30 *m*	3.30 *m*	3.36 nr
6″	3.73 *dd* (12.3, 2.4)	3.73 *dd* (12.2, 2.5)	3.69 nr
	3.67 *dd* (12.3)	3.67 *dd* (12.2, 4.6)	3.69 nr
Shikimic acid moiety			
2‴	–	6.68 *br s*	6.65 *br s*
3‴	–	4.09 *br s*	4.06 *br s*
4‴	–	3.71 *br s*	3.72 nr
5‴	–	5.05 *dd* (9.8, 4.0)	5.04 *dd* (9.9, 4.1)
6‴	–	2.56 *br d* (19.0)	2.56 *br d* (18.3)
	–	2.17 *br d* (19.0)	2.14 *br d* (18.3)
Heptitol moiety			
1⁗	–	–	4.40 *dd* (11.8, 2.8)
	–	–	4.28 *dd* (11.8, 6.9)
2⁗	–	–	4.06 *br s*
3⁗	–	–	3.80 nr
4⁗	–	–	3.80 nr
5⁗	–	–	3.90 *dd* (14.3, 4.6)
6⁗	–	–	3.80 nr
7⁗	–	–	3.69 nr
	–	–	3.62 *dd* (11.5, 6.0)
Methyl ester	3.58 *s*	–	–

one of the two remaining carboxy groups of subunit **2**. The HMBC clearly indicated a bond to C-9 of the lignan, explaining the highfield shift of this carbon atom compared to compound **2**. Additional proof of the structure gave a LC-ESI mass spectrum with the mass of 870 amu, consistent with the molecular formula of $C_{38}H_{46}O_{23}$, giving the new epiphyllic acid-7-*O*-β-glucoside-9,1⁗-*O*-heptitol ester-10,5‴-*O*-shikimic acid ester.

The stereochemistry of the heptitol moiety, however, remains to be clarified. There exist ^{13}C NMR data of free heptitols (Angyal & Le Fur, 1984), but since the ^{13}C NMR data depend on the conformation adopted in solution and the ester bond alters both the values of C-1⁗ and C-2⁗ and probably the conformation, no conclusion can be drawn. Unfortunately, the amount isolated was too small for further chemical experiments.

The structure elucidation of **4** proved to be rather simple. Besides the structure of the lignan moiety, two shikimic acid moieties were present with one shikimic acid moiety having resonances shifted downfield in the ^{1}H NMR spectrum and being merely unchanged in the ^{13}C NMR spectrum (Table 3). From these data, two 5-*O*-shikimic acid esters bound to the two carboxylic carbon atoms of the lignan moiety were assumed, giving identical ^{13}C NMR data and the ^{1}H NMR differences being only the result of some anisotropic fields. From the results of the HMBC spectrum, the downfield shifted shikimic acid moiety could be attributed to C-9 and consequently the shikimic acid with data similar to those of compounds **2** and **3** showed a correlation to C-10. Therefore, **4** is the new epiphyllic acid-9,5‴-O-,10,5⁗-*O*-bis(shikimic acid ester).

Another detail is worth mentioning. Upon binding of greater molecules to the carboxylic carbon atoms of the lignan moiety, the coupling constant between H-1 and H-2 increases. The free lignan from *P. epiphylla* (Cullmann et al., 1993) showed a coupling constant of 2.5 Hz, compared to compounds **1**–**3**, which have



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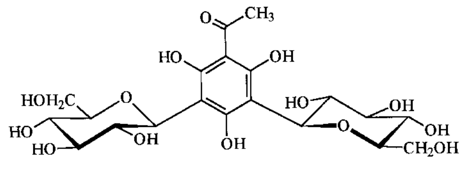

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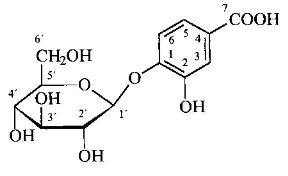

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values of about 4.5 Hz. Compound **4** even has $J = 8.0$ Hz. Additionally, there are small differences in the ^{13}C NMR data of the carbon atoms being next to these two centres. All these findings suggest a conformational change upon substitution leading to a structure, where these two *trans* substituted protons adopt a more axial-axial conformation to allow both the phenyl ring and the substituent at C-10 to be in the energetic favoured equarorial position.

From the results of our studies (Cullmann et al., 1996; 1999; Martini et al., 1998; Tazaki et al., 1995), only a few conclusions can be drawn. First, up to now, lignans occur only in the Jungermanniideae and have not been found in the Marchantiideae. Within the Jungermanniideae, they were found both in the Metzgeriales (*P. epiphylla* (Cullmann et al., 1993; 1996)) and the Jungermanniales (*Jamesionella autumnalis* (Tazaki et al., 1995), *Lepidozia reptans* (Sanders, 1996), *Bazzania trilobata* (Martini et al., 1998) *Lepidozia incurvata, Chiloscyphus polyanthos*, and *Jungermannia exsertifolia* (Cullmann et al., 1999)). The key structure and parent compound of all lignans seems to be epiphyllic acid, derived from two caffeic acid moieties. Either oxidation, unsaturation and decarboxylation occur to give other basic structures, or the epiphyllic acid is unchanged and the lignans undergo glycosidation or esterification to form more complex and even unique structures. So far, no correlation between specific structures and taxonomic classification is obvious. This, of course, results from the small number of liverworts examined so far. Further work is in progress.

Table 2
^{13}C NMR spectral data for compounds **1**, **2**, and **3** (methanol-d_4)

C	**1**	**2**	**3**
Lignan moiety			
1	47.2 *d*	47.4 *d*	47.2 *d*
2	48.8 *d*	49.4 *d*	49.3 *d*
3	126.1 *s*	128.3 *s*	125.1 *s*
4	138.6 *d*	138.7 *d*	139.3 *s*
4a	128.3 *s*	126.0 *s*	128.0 *s*
5	117.5 *d*	117.5 *d*	117.7 *d*
6	147.5 *s*	147.5 *s*	147.3 *s*
7	148.3 *s*	148.3 *s*	148.5 *s*
8	118.9 *d*	118.7 *d*	118.6 *d*
8a	131.1 *s*	131.0 *s*	131.1 *s*
9	170.2 *s*	170.1 *s*	168.5 *s*
10	175.2 *s*	173.8 *s*	173.8 *s*
1′	135.6 *s*	135.2 *s*	135.1 *s*
2′	115.9 *d*	116.0 *d*	116.0 *d*
3′	146.2 *s*	146.2 *s*	146.1 *s*
4′	145.2 *s*	145.3 *s*	145.2 *s*
5′	116.3 *d*	116.4 *d*	116.5 *d*
6′	120.1 *d*	120.3 *d*	120.3 *d*
Methyl ester	52.7 *q*	–	–
Glucose moiety			
1″	103.7 *d*	103.6 *d*	103.4 *d*
2″	74.8 *d*	74.8 *d*	74.7 *d*
3″	77.6 *d*	77.5 *d*	77.4 *d*
4″	71.0 *d*	71.0 *d*	70.9 *d*
5″	78.1 *d*	78.0 *d*	77.9 *d*
6″	62.1 *t*	62.1 *t*	62.0 *t*
Shikimic acid moiety			
1‴	–	129.4 *s*	129.5 *s*
2‴	–	139.3 *d*	139.3 *d*
3‴	–	67.1 *d*	67.1 *d*
4‴	–	68.6 *d*	68.6 *d*
5‴	–	72.2 *d*	72.4 *d*
6‴	–	27.4 *t*	27.6 *t*
7‴	–	169.5 *s*	169.9 *s*
Heptitol moiety			
1⁗			67.4 *t*
2⁗			72.3 *d*
3⁗			73.2 *d*[a]
4⁗			74.1 *d*[a]
5⁗			71.1 *d*
6⁗			74.9 *d*
7⁗			64.1 *t*

[a] Signals may be interchanged.

3. Experimental

Solvents used for spectral measurements: MeOH-d_4 (^1H NMR: 400 MHz; ^{13}C NMR: 100 MHz for 1D,

Table 3
^{1}H and ^{13}C NMR spectral data and coupling constants (in Hz in parentheses) for compound **4** (methanol-d_4)

H	**4**	C	**4**
Lignan moiety		Lignan moiety	
1	4.19 *d* (8.0)	1	48.1 *d*
2	3.65 *d* (8.0)	2	49.5 *d*
4	7.40 *s*	3	124.3 *s*
5	6.67 *s*	4	140.8 *d*
8	6.36 *s*	4a	125.0 *s*
2′	6.54 *d* (2.0)	5	117.8 *d*
5′	6.60 *d* (8.1)	6	144.9 *s*
6′	6.43 *dd* (8.1, 2.0)	7	148.9 *s*
Shikimic acid moiety 1		8	116.4 *d*
2″	6.49 *br s*	8a	132.2 *s*
3″	3.73 *m*	9	167.9 *s*
4″	3.46 *dd* (4.4, 4.4)	10	172.6 *s*
5″	4.88 *m*	1′	132.5 *s*
6″	2.34 *br d* (18.4)	2′	117.7 *d*
	1.97 *br d* (18.4)	3′	146.3 *s*
Shikimic acid moiety 2		4′	145.7 *s*
2‴	6.77 *br s*	5′	116.5 *d*
3‴	4.25 *br s*	6′	122.0 *d*
4‴	3.73 *m*	Shikimic acid moiety 1	
5‴	5.10 *dd* (9.7, 5.0)	1″	129.1 *s*
6‴	2.69 *br d* (18.4)	2″	139.7 *d*
	2.20 *dd* (18.4, 5.0)	3″	67.0 *d*
		4″	68.1 *d*
		5″	72.1 *d*
		6″	26.9 *t*
		7″	169.7 *s*
		Shikimic acid moiety 2	
		1‴	130.3 *s*
		2‴	138.8 *d*
		3‴	67.3 *d*
		4‴	70.1 *d*
		5‴	71.8 *d*
		6‴	29.2 *t*
		7‴	169.7 *s*

500 and 125 MHz for 2D techniques, respectively. Chemical shifts are given in δ values from TMS), MeOH (optical rotation).

LC-MS was performed on a HP API-ES LC-MS with a HP59987A electrospray interface and a HP 5989B MS engine.

3.1. Plant material

L. ochroleuca (Spreng.) Spruce (400 g) was collected in March 1997, 18 km East of Puerto Cisnes, Chile and identified by Prof. Dr R. Mues. Voucher specimen is deposited in the Herbarium Mues (No. 3409).

3.2. Extraction and isolation

Air dried *L. ochroleuca* was sequentielly extracted with Et_2O and MeOH. The MeOH extract was evpd. in vacuo and partitioned between EtOAc and H_2O. The aqueous layer was again distributed between ButOH and H_2O. The organic layer was evpd. in vacuo and chromatographed on Sephadex LH20 using MeOH as eluent to yield three main frs. Fr. I was separated by HPLC on RP18 (MeOH–H_2O 20:80 + 1% HCOOH) to give **3** (26.1 mg) and **5** (11.9 mg). Fr. II was chromatographed likewise (MeOH–H_2O 30:70 + 1% HCOOH) to yield **6** (7.5 mg), **2** (17.4 mg), and **1** (13.6 mg). From Fr. III, compound **4** (45.9 mg) was isolated in the same manner (MeOH–H_2O 40:60 + 1% HCOOH).

3.2.1. Compound **1**

$[\alpha]_D^{20} = -124.1$ (c 0.66). ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. LC-MS: m/z 573 $[M+K]^+$, 535 $[M]^+$.

3.2.2. Compound **2**

$[\alpha]_D^{20} = -137.4$ (c 0.82). ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. LC-MS: m/z 715 $[M+K]^+$.

3.2.3. Compound **3**

$[\alpha]_D^{20} = -119.1$ (*c* 0.38). ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. LC-MS: *m/z* 871 $[M+H]^+$.

3.2.4. Compound **4**

$[\alpha]_D^{20} = -243.6$ (*c* 0.70). ^{1}H NMR: Table 3. ^{13}C NMR: Table 3. LC-MS: *m/z* 708 $[M+K]^+$, 671 $[M+H]^+$.

3.2.5. Compound **5**

$[\alpha]_D^{20} = +65.2$ (*c* 0.21) (had not been published yet).

3.2.6. Compound **6**

$[\alpha]_D^{20} = -75.0$ (*c* 0.38). ^{1}H NMR: 7.50 (*dd*, 8.4, 2.0, H-5), 7.47 (*d*, 2.0, H-3), 7.22 (*d*, 8.4. H-6), 4.90 (*d*, 7.4, H-1′), 3.90 (*dd*, 12.0, 2.0, H-6′), 3.71 (*dd*, 12.0, 5.4, H-6′), 3.55-3.38 (3H, *m*, H-2′, H-3′, H-4′), 3.30 (*m*, H-5′). CIMS: *m/z* 317 $[M+H]^+$.

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