Phytochemistry 80 (2012) 115–122

Contents lists available at SciVerse ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Unusual terpenylated acylphloroglucinols from *Dryopteris wallichiana*

Cecilia Socolsky [a,b,*], Laura Domínguez [c], Yoshinori Asakawa [b], Alicia Bardón [a]

[a] INQUINOA – CONICET, Ayacucho 471, Tucumán 4000, Argentina
[b] Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan
[c] Universidad de la República Oriental del Uruguay, General Flores 2124, Montevideo 11800, Uruguay

ARTICLE INFO

Article history:
Received 23 January 2012
Received in revised form 16 March 2012
Accepted 24 April 2012
Available online 1 June 2012

Keywords:
Dryopteris wallichiana (Spreng.) Hyl.
Dryopteridaceae
Terpenylated acylphloroglucinols
Nematocidal activity
Nippostrongylus brasiliensis

ABSTRACT

Four unusual terpenylated acylphloroglucinols were isolated from the diethyl ether extract of the scales and rhizomes of the fern *Dryopteris wallichiana* together with the known compounds albaspidins AA and AB, and filixic acids ABA and ABB. Structures of the isolated compounds were established by extensive spectroscopic analysis and their absolute configuration at C-14″ was determined by comparing their CD spectra with those simulated for the respective isomers. Pure acylphloroglucinols displayed moderate *in vitro* nematocidal activity against L4 stage larvae of *Nippostrongylus brasiliensis* (LD_{50} = 22–121 μM).

1. Introduction

Powdered rhizomes and extracts of ferns belonging to the genus *Dryopteris*, particularly those of the male fern (*D. filix-mas*), were used in the past as effective anthelmintic agents; their effects are related to the presence of mixtures of acylphloroglucinols in these plants (Chopra et al., 1985; Murakami and Tanaka, 1988). The isolation of the bioactive compounds started in the 19th century due to the strong biological activity of the extracts. So far, a large number of *Dryopteris* species have been analyzed, and many acylphloroglucinols with two up to six rings have been characterized (Widén et al., 1994). However, the structures of several compounds isolated from extracts of various *Dryopteris* species could not be completely elucidated. From some of these incomplete structural descriptions (Euw et al., 1980; Fraser-Jenkins and Widén, 1993; Widén et al., 1991), it might be inferred that a terpenyl moiety is attached to an acylphloroglucinol residue. For this reason, the reinvestigation of the extracts for these unusual metabolites from some *Dryopteris* species is of great interest.

Terpenylated acylphloroglucinols are rare in *Dryopteris* ferns. Pentherin-I, a sesquiterpenylated flavaspidic acid, was isolated from *D. pentheri* in 1973 (Widén et al., 1973) although its structure could only be elucidated in part. The structural elucidation of the first terpenylated phloroglucinol derivatives from the fronds of *D. atrata* was reported 24 years later (Fuchino et al., 1997). They consisted of a norflavaspidic acid AA or AB linking an aristolene-type sequiterpenoid at C-5′. *Dryopteris wallichiana* was investigated repeatedly in search for acylphloroglucinols (Hisada and Noro, 1961; Tryon et al., 1973; Widén et al., 1996). Although many acylphlorolgucinol dimers, trimers and tetramers were isolated/detected, specimens of *D. wallichiana* ssp. *wallichiana* from India contained unknown phenolic compounds closely related to pentherin-I, whereas herbarium material from Mexico contained phloroglucinol derivatives Wa-1 and 2, whose structures could not be fully characterized (Widén et al., 1996).

Continuing with our search for bioactive acylphloroglucinols from ferns (Socolsky et al., 2009, 2010a,b, 2011a,b), reported herein is the isolation of four new unique terpenylated phloroglucinol derivatives along with four known compounds from the scales and rhizomes of an Argentine collection of the fern *D. wallichiana* (Spreng.) Hyl. This is the second report on structure elucidation of terpenylated acylphloroglucinols from *Dryopteris* ferns. These new compounds are as abundant as the known acylphloroglucinols in the crude filicin (diethyl ether extract) of this collection of *D. wallichiana* (Section 4.3). Because terpenylated acylphloroglucinols might also be responsible for the anthelmintic effects of *Dryopteris*' extracts, the *in vitro* nematocidal activity of pure acylphloroglucinols was evaluated against larvae of *Nippostrongylus brasiliensis* at their parasitant stage L4.

2. Results and discussion

2.1. Isolation and structure elucidation

The scales and rhizomes of *D. wallichiana* were ground and extracted with Et_2O. The resulting extract was processed on silica gel

* Corresponding author at: INQUINOA – CONICET, Ayacucho 471, Tucumán 4000, Argentina. Tel.: +54 381 4247752; fax: +54 381 4248169.
E-mail address: csocolsky@fbqf.unt.edu.ar (C. Socolsky).

0031-9422/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2012.04.017

and Sephadex LH-20 to obtain two acylphloroglucinol-containing fractions (TLC analysis), that were purified by normal phase HPLC to afford four acylphloroglucinols of novel structure named wallichins A–D (**1**–**4**, Fig. 1). The known compounds filixic acids ABA and ABB were also isolated, along with albaspidins AA and AB.

Wallichin A (**1**) was obtained as a yellow gum. Its molecular formula, $C_{42}H_{54}O_{10}$, that accounts for 16° of unsaturation, was deduced from a molecular ion peak observed at *m/z* 718.3723 (calcd. 718.3718) in its HREIMS spectrum. The IR spectrum of this compound showed a broad absorption centered at 3230 cm^{-1} assigned to the O–H stretching of a hydroxyl group involved in intramolecular hydrogen bonding, an intense band at 1693 cm^{-1} attributed to conjugated carbonyl groups, as well as other intense bands at 1639 and 1614 cm^{-1} assigned to an aromatic ring. The ^{1}H NMR spectrum of compound **1** in acetone-d_6 (Table 1) showed a singlet at a very low field (δ 18.57, 5-OH), assigned to an enolizable β-triketonic system. This evidence together with signals for a *gem*-dimethyl group at δ 1.52 and 1.50 (3H each, *s*) established the presence of an acylfilicinic acid ring. This was corroborated by comparison with literature data (Lounasmaa, 1978; Wollenweber et al., 1998). Two doublets arising from an AB system were observed at δ 3.48 and 3.45 (1H each, *d*, *J* = 16.4 Hz) in the ^{1}H NMR spectrum of **1** suggesting the presence of a methylene bridge whose chemical shift indicated that the CH_2 connected an acylfilicinic acid and an aromatic ring (v. Euw et al., 1985). NMR spectroscopic data also provided evidence of acetyl ($C\underline{H}_3CO$, δ_H 2.70, 3H, *s*) and butyryl (δ_H 3.16 and 3.12, 1H each, *dt*, *J* = 16.6, 7.3 Hz, 1.70, 2H, *sext.*, *J* = 7.3 Hz; and 0.98, 3H, *t*, *J* = 7.3 Hz) groups attached to the rings. Their location was established through long range H–C correlations in the HMBC spectrum of **1** (Fig. 2). Crosspeaks observed between H-11 and C-6, as well as between 4′-OH and C-8′, established the position of the acetyl group at C-6 on the filicinic acid ring, and the butyryl residue at C-3′ on the acylphloroglucinol-type ring.

The presence of a terpenyl moiety was evident from analysis of the ^{1}H and ^{13}C NMR spectra of **1** (Table 1) while HMBC correlations between CH_2-7′ and C-4′, C-5′, and C-6′ indicated the position of the sidechain at C-5′ (Fig. 2). Substraction of the contributions of the acylphloroglucinol moiety from the molecular formula of **1** gave the subformula $C_{21}H_{32}O_2$ for the terpenyl substituent. Ten unsaturations accounted for the acylphloroglucinol residue. Therefore, the terpenyl residue should have the remaining six. The ^{1}H NMR spectrum of **1** showed the presence of a CH_2 of an exocyclic double bond (δ 4.86, 1H, *d*, *J* = 1.2 Hz, H-17″a and δ 4.62, 1H, *d*, *J* = 1.2 Hz, H-17″b) and a trisubstituted double bond (δ 5.66, 1H, br. *t*, *J* = 6.3 Hz, H-12″). The signal at δ 179.7 ppm on the ^{13}C NMR spectrum of **1** established the presence of a carboxyl group (C-18″). Then, the terpenyl moiety should have three cycles. Cyclization of the sidechain to form a pyrane ring (chromane) was evident from the ^{13}C NMR signal at δ 85.0 ppm, assigned to C-14″. The chemical shift of the 4′-OH (δ 14.12 ppm) indicated a strong association by hydrogen bonding to the carbonyl group at C-3′, which was confirmed by an HMBC correlation observed between this hydroxyl group and C-8′ (Fig. 2). Moreover, only one OH group attached to the aromatic ring (OH-2′) showed a correlation with C-1′ (Fig. 2). Thus, cyclization should occur at C-6′, and comparison with literature data supported this deduction (Ishiguro et al., 1994; Rocha et al., 1996; Socolsky et al., 2010a,b). Spectroscopic evidence (Table 1) and comparison with literature data allowed us to identify the terpenyl moiety as a labda-8(17),12*E*-dien-18-oic acid (Kuo et al., 2008; Minami et al., 2002). The *E* configuration of the double bond at C-12″ was established through a NOESY correlation observed between H-11″ and H-16″ (Fig. 3). Other key NOESY correlations were observed between H-20″ and H-19″, as well as between H-5″ and H-9″, indicating the relative stereochemistry of the terpenyl moiety (Fig. 3). The *ent*- configuration for the labdane-type moiety is thus proposed, since usually the mentioned configuration has been reported for fern labdanes (Murakami and Tanaka, 1988; Socolsky et al., 2007). Additional evidence supporting the proposed structure was obtained from the EIMS spectrum of **1** which showed ions of *m/z* 208 (base peak) and 510, both originating from the molecular ion by hydrogen transfer with cleavage of the methylene bridge (Fig. 4). This rearrangement usually yields the most intense peaks in the MS spectrum of *Dryopteris*' acylphloroglucinols and is observed when using different ionization techniques, such as EI, FAB, CI, and APCI (Lounasmaa, 1979; Lounasmaa et al., 1972; Widén et al., 1992, 1994; Wollenweber et al., 1998).

The CD spectrum of **1** showed positive Cotton effects at $\Delta\varepsilon_{206}$ + 23.40, $\Delta\varepsilon_{299}$ + 1.53, and $\Delta\varepsilon_{332}$ + 0.72 and negative Cotton effects at $\Delta\varepsilon_{228}$ − 12.05 and $\Delta\varepsilon_{361}$ − 0.89. The absolute configuration

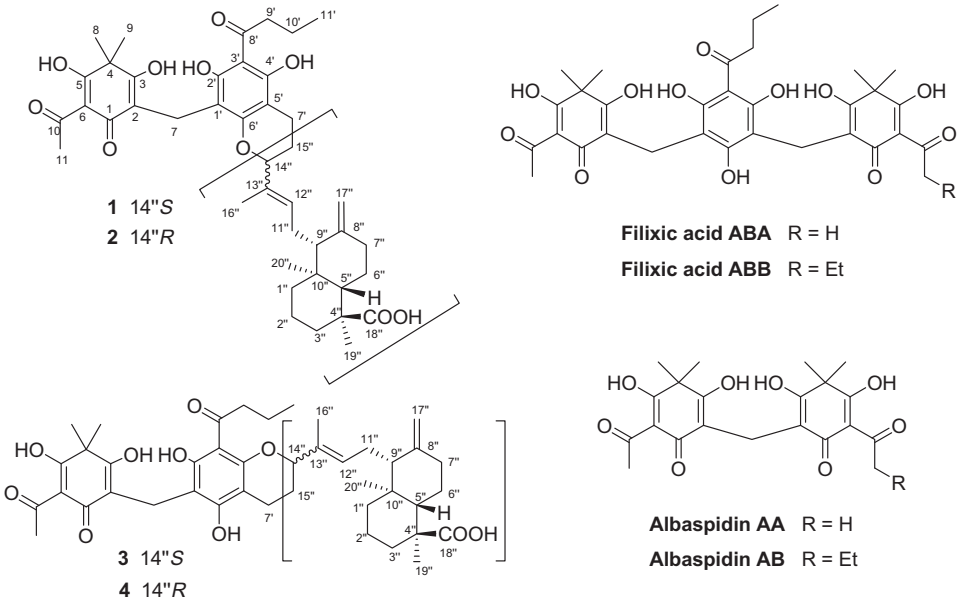

Fig. 1. Structure of terpenylated acylphloroglucinols and known compounds isolated from the Argentine collection of *D. wallichiana*.

Table 1
Spectroscopic data of compounds **1** and **2** (acetone-d_6, 600 MHz).

	1			**2**		
Position	δ ^{1}H (*mult.*, *J* in Hz)	δ_C (m)	HMBC (^{1}H → ^{13}C)	δ ^{1}H (*mult.*, *J* in Hz)	δ_C (m)	HMBC (^{1}H → ^{13}C)
1		188.4 s			188.4 s	
2		112.1 s			112.1 s	
3		172.2 s			172.1 s	
4		44.9 s			44.9 s	
5		199.7 s			199.7 s	
6		109.2 s			109.2 s	
7	3.48 (*d*, 16.4)	17.6 t	1, 2, 3, 1′, 2′, 6′	3.48 (*d*, 15.9)	17.7 t	1, 2, 3, 1′, 2′, 6′
	3.45 (*d*, 16.4)		1, 2, 3, 1′, 2′, 6′	3.45 (*d*, 15.9)		1, 2, 3, 1′, 2′, 6′
8	1.52 (*s*)	24.9 q	3, 4, 5, 9	1.54 (*s*)	25.0 q	3, 4, 5, 9
9	1.50 (*s*)	25.1 q	3, 4, 5, 8	1.51 (*s*)	25.2 q	3, 4, 5, 8
10		204.4 s			204.4 s	
11	2.70 (*s*)	29.3 q	6, 10	2.70 (*s*)	29.3 q	6, 10
1′		105.0 s			105.1 s	
2′		158.7 s			158.7 s	
3′		106.7 s			106.7 s	
4′		163.2 s			163.2 s	
5′		102.7 s			102.7 s	
6′		158.2 s			158.4 s	
7′	2.72 (*ddd*, 16.7, 5.7, 3.1)	19.1 t	4′, 5′, 6′, 14″,15″	2.75 (*ddd*, 16.5, 5.0, 3.1)	19.2 t	4′, 5′, 6′, 15″
	2.50 (*ddd*, 16.7, 11.3, 5.7)		4′, 5′, 6′, 14″,15″	2.55–2.47[a]		4′, 5′, 6′, 15″
8′		207.7 s			207.8 s	
9′	3.16 (*dt*, 16.6, 7.3)	46.9 t	8′, 10′, 11′	3.17 (*dt*, 16.6, 7.3)	46.9 t	8′, 10′, 11′
	3.12 (*dt*, 16.6, 7.3)		8′, 10′, 11′	3.14 (*dt*, 16.6, 7.3)		8′, 10′, 11′
10′	1.70 (*sextet*, 7.3)	18.8 t	8′, 9′, 11′	1.70 (*sextet*, 7.3)	18.8 t	8′, 9′, 11′
11′	0.98 (*t*, 7.3)	14.5 q	9′, 10′	0.98 (*t*, 7.3)	14.2 q	9′, 10′
1″	1.90–1.83[a]	39.1 t	2″, 3″, 9″, 10″, 20″	1.93 (*br dt*, 12.8)	39.2 t	3″, 9″, 10″, 20″
	1.24 (*td*, 12.8, 3.9)		2″, 3″, 9″, 10″, 20″	1.28 (*td*, 12.8, 4.1)		3″, 9″, 10″, 20″
2″	1.70–1.58[a]	19.2 t	ND	1.65 (*ddd*, 18.1, 6.7, 3.2)	19.2 t	ND
	1.70–1.58[a]		ND	1.70–1.59[a]		ND
3″	1.80 (*td*, 12.4, 4.3)	37.8 t	1″, 2″, 4″, 5″, 19″	1.82 (*td*, 12.6, 4.1)	37.8 t	2″, 4″, 18″, 19″
	1.64–1.58[a]		1″, 2″, 4″, 5″, 19″	1.65–1.59[a]		2″, 4″, 18″, 19″
4″		47.7 s			47.8 s	
5″	2.04 (*dd*, ND, 2.5)	50.4 d	4″, 6″, 7″, 9″, 10″, 18″, 19″, 20″	2.08 (*dd*, 12.8, 2,7)	50.5 d	4″, 6″, 7″, 9″, 10″, 18″, 19″, 20″
6″	1.47 (*td*, 12.8, 4.3)	27.3 t	5″, 7″, 10″	1.48 (*td*, 12.8, 4.2)	27.3 t	7″
	1.38 (*ddt*, 12.8, 5.3, 2.5)		5″, 7″, 10″	1.38 (*ddd*, 12.8, 5.5, 2.7)		7″
7″	2.36 (*ddd*, 12.8, 4.3, 2.5)	38.4 t	8″, 9″	2.38 (*ddd*, 12.8, 4.2, 2.7)	38.4 t	5″, 6″, 8″, 9″, 17″
	2.06–2.00[a]		8″, 9″	2.10–2.05[a]		5″, 6″, 8″, 9″, 17″
8″		148.7 s			148.9 s	
9″	1.90 (*br d*, 10.1)	57.9 d	5″, 8″, 10″, 11″, 12″, 17″, 20″	2.03–1.96[a]	58.1 d	5″, 8″, 10″, 11″, 20″
10″		39.5 s			39.6 s	
11″	2.40 (*br dd*, 15.6, 6.3)	23.3 t	8″, 9″, 12″/13″	2.55–2.47[a]	23.2 t	8″, 9″, 12″, 13″
	2.25 (*ddd*, 15.6, 11.0, 6.3)		8″, 9″, 12″/13″	2.18 (*ddd*, 15.1, 11.1, 5.9)		8″, 9″, 12″, 13″
12″	5.66 (*br t*, 6.3)	132.8 d	9″, 11″, 14″, 16″	5.73 (*br t*, 6.3)	132.7 d	9″, 11″, 14″, 16″
13″		132.8 s			133.5 s	
14″	4.63 (*dd*, 10.1, 2.0)	85.0 d	7′, 12″/13″, 15″, 16″	4.61 (*dd*, 9.5, 3.3)	85.4 d	7′, 13″, 15″, 16″
15″	2.06–1.96[a]	26.0 t	5′, 7′	2.03–1.96[a]	25.8 t	ND
16″	1.85 (*br s*)	12.6 q	12″/13″, 14″	1.85 (*br s*)	12.2 q	12″, 13″, 14″
17″	4.86 (*d*, 1.2)	108.6 t	7″, 8″, 9″	4.86 (*d*, 1.4)	108.2 t	7″, 8″, 9″
	4.62 (*d*, 1.2)		7″, 8″, 9″	4.59 (*d*, 1.4)		7″, 8″, 9″
18″		179.7 s			179.6 s	
19″	1.15 (*s*)	17.1 q	3″, 4″, 5″, 18″	1.15 (*s*)	17.1 q	3″, 4″, 5″
20″	0.81 (*s*)	15.0 q	1″, 5″, 9″, 10″	0.81 (*s*)	15.0 q	1″, 5″, 9″, 10″
3-OH	9.10 (*s*)	–	2, 3, 4	9.04 (*s*)	–	2, 3, 4
5-OH	18.57 (*s*)	–	4, 5, 6, 10	18.56 (*s*)	–	4, 5, 6, 10
2′-OH	11.43 (*s*)	–	1′, 2′, 3′	11.47 (*s*)	–	1′, 2′, 3′
4′-OH	14.12 (*s*)	–	3′, 4′, 5′, 8′	14.13 (*s*)	–	3′, 4′, 5′

[a] Overlapping signals, ND: Not determined due to overlap with solvent or other signals.

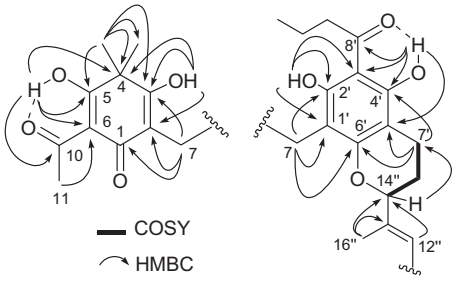

Fig. 2. Some key HMBC and COSY correlations of wallichin A (**1**).

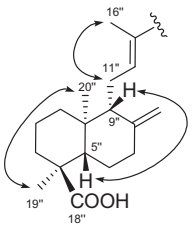

Fig. 3. Key NOE correlations of the terpenyl moiety of wallichin A (**1**).

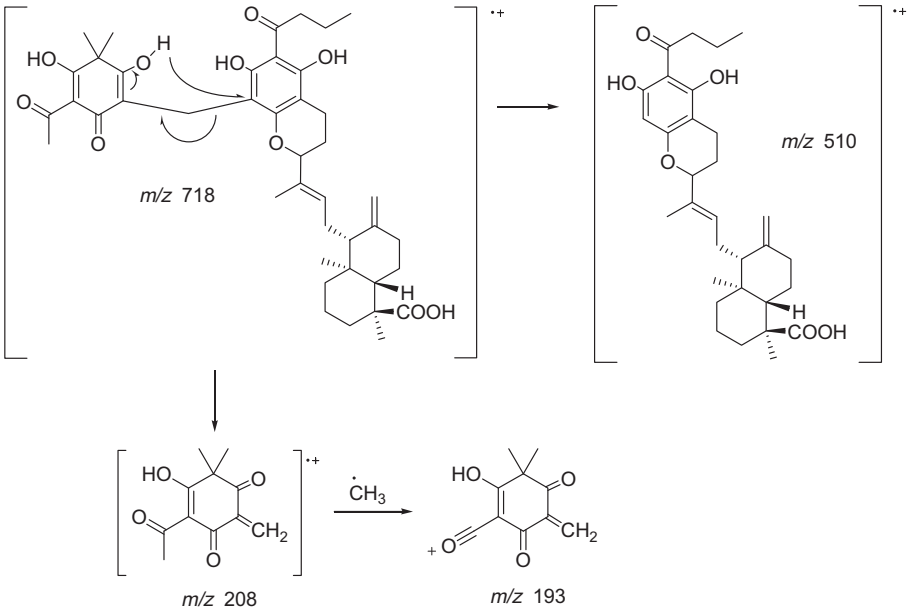

Fig. 4. Some EIMS fragmentations of wallichin A (**1**).

of compound **1** at C-14″ was assessed by simulating its electronic circular dichroism spectrum (ECD) using the time-dependent density functional theory (TD-DFT) method (Jiemchooroj and Norman, 2007; Wu et al., 2011). On the basis of the absolute configuration of the terpenyl moiety, two hypothetical molecules were modeled with configurations *R* and *S* at C-14″. Geometries of the mentioned molecules in ethanol solution were optimized at the B3LYP/6–31G* level of theory. Then, their ECD spectra were calculated with the same combination of method and basis set at the optimized geometries (Supporting Information, Table S1). The calculated ECD spectrum of the 14″*S* isomer gave the best fit with the experimental CD of **1** (Fig. 5). Based on the foregoing evidence, the structure of wallichin A (**1**) could be established as depicted.

Wallichin B (**2**) has the same molecular formula of compound **1**, and thus they are isomers. The ^{1}H and ^{13}C NMR spectroscopic data of **2** (Table 1) were similar to those of compound **1** and only slight differences were observed in their 1D NMR spectra, particularly in the signals of atoms near C-14″. The $[\alpha]_D$ of this compound was found to be −27.1, while the corresponding value for wallichin A (**1**) is +23.6. The intensities of the main peaks in the EIMS spectrum of **2** were significantly different from the corresponding signals in that of **1**, indicating that these two compounds were neither identical nor enantiomers (Sections 4.3.1 and 4.3.2). Based on the presented evidence, and due to the fact that cyclization of the chromane could take place to give any of the two epimers, wallichin B (**2**) might be an epimer of acylphloroglucinol **1** at C-14″. In order to clarify this point, the CD spectrum of **2** was recorded. It showed bands of opposite sign when compared with that of **1** (Fig. 6). These two compounds behave as enantiomers by CD, probably due to the

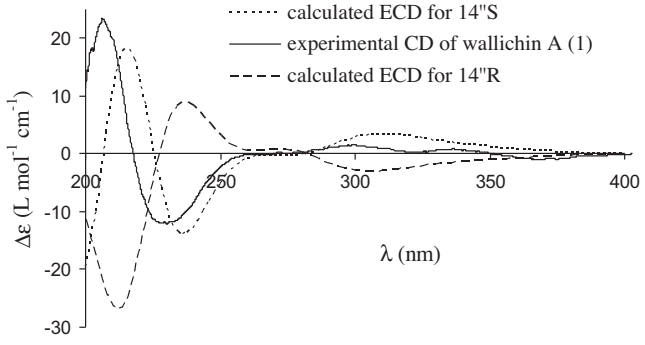

Fig. 5. Calculated ECD spectra of 14″*R* and 14″*S* isomers of compounds **1** compared with the experimental CD spectrum of wallichin A (**1**) in ethanol solution.

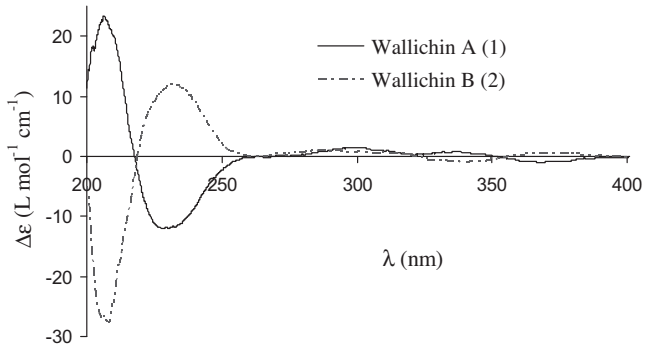

Fig. 6. Comparison of the experimental CD spectra of compounds **1** and **2** in ethanol.

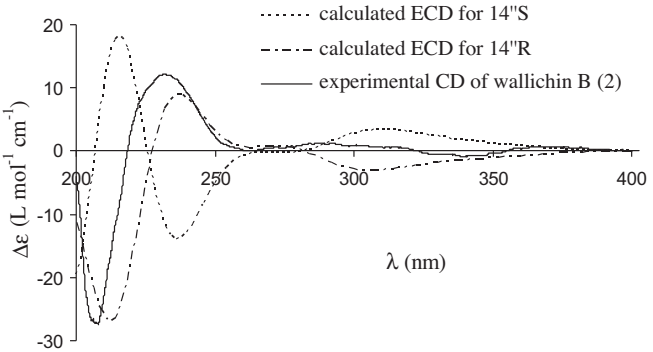

Fig. 7. Experimental CD spectrum of **2** in ethanol solution compared with simulated ECD spectra of both epimers at C-14″ (14″*S* and 14″*R*).

fact that C-14″ is the only chiral center near the chromophores. The absolute configuration at C-14″ was established as *R* by comparison with the simulated ECD for this epimer (Fig. 7).

The molecular formula of wallichin C (**3**), $C_{42}H_{54}O_{10}$, was obtained from its HREIMS spectrum (*m/z* 718.3706, calcd 718.3718). This compound has the same molecular formula of **1** and **2**. The 1D NMR spectroscopic data of **3** (Table 2) resembled those of **1** and **2**. The main difference concerned the chemical shift of the hydroxyl protons of the acylphloroglucinol-type ring, OH-2′ and OH-6′ (δ 16.48 and 11.26, respectively), in the ^{1}H NMR spectrum of compound **3**. As was previously reported for other acylphloroglucinols, the observed chemical shifts indicated that the cyclization pattern involves the OH group at C-4′ instead of that at C-6′ (Ishiguro et al., 1994; Rocha et al., 1996; Socolsky et al., 2010b). Further evidence of the cyclization pattern was obtained from the HMBC spectrum of **3** that showed correlations of the two OH groups attached to the aromatic ring (OH-2′ and OH-6′) with C-1′. Thus, the structure of wallichin C (**3**) was established as shown. To determine the absolute configuration of **3** at C-14″, the ECD spectra of both epimers were simulated. The isomer with *S* configuration gave the best fit with the experimental CD spectrum (Fig. 8).

Table 2
NMR spectroscopic data of compounds **3** and **4** (acetone-d_6, 600 MHz).

	3			**4**		
Position	δ ^{1}H (*mult.*, *J* in Hz)	δ_C (m)	HMBC (^{1}H → ^{13}C)	δ ^{1}H (*mult.*, *J* in Hz)	δ_C (m)	HMBC (^{1}H → ^{13}C)
1		188.3 s			188.3 s	
2		112.1 s			112.1 s	
3		172.6 s			172.6 s	
4		45.0 s			45.0 s	
5		199.9 s			199.9 s	
6		109.1 s			109.2 s	
7	3.53 (*d*, 15.7)	17.2 t	1, 2, 3, 1′, 2′, 6′	3.53 (*d*, 15.8)	17.2 t	1, 2, 3, 1′, 2′, 6′
	3.50 (*d*, 15.7)		1, 2, 3, 1′, 2′, 6′	3.50 (*d*, 15.8)		1, 2, 3, 1′, 2′, 6′
8	1.478 (*s*)	24.9 q	3, 4, 5, 9	1.478 (*s*)	24.9 q	3, 4, 5, 9
9	1.482 (*s*)	25.0 q	3, 4, 5, 8	1.485 (*s*)	25.0 q	3, 4, 5, 8
10		204.2 s			204.2 s	
11	2.70 (*s*)	29.2 q	6, 10	2.70 (*s*)	29.2 q	6, 10
1′		106.0 s			106.0 s	
2′		161.6 s			161.6 s	
3′		104.8 s			104.8 s	
4′		158.4 s			158.4 s	
5′		103.8 s			103.8 s	
6′		162.6 s			162.6 s	
7′	2.73 (*ddd*, 16.7, 5.5, 2.5)	20.2 t	4′, 5′, 14″, 15″	2.74 (*ddd*, 16.7, 5.7, 2.5)	20.2 t	4′, 5′, 6′, 15″
	2.54 (*ddd*, 16.7, 11.7, 5.5)		4′, 5′, 14″, 15″	2.55 (*ddd*, 18.2, 11.9, 6.5)		4′, 5′, 6′, 15″
8′		207.2 s			207.2 s	
9′	3.02 (*ddd*, 15.8, 8.4, 6.6)	46.5 t	8′, 10′, 11′	3.02 (*ddd*, 16.2, 7.9, 6.9)	46.4 t	8′, 10′, 11′
	2.92 (*ddd*, 15.8, 8.4, 6.6)		8′, 10′, 11′	2.95 (*ddd*, 16.2, 7.9, 6.9)		8′, 10′, 11′
10′	1.70–1.57[a]	19.2 t	9′, 11′	1.70–1.57[a]	19.0 t	8′, 9′, 11′
11′	0.96 (*t*, 7.3)	14.5 q	9′, 10′	0.95 (*t*, 7.3)	14.3 q	9′, 10′
1″	1.91–1.84[a]	39.1 t	2″, 3″, 9″, 10″, 20″	1.92–1.82[a]	39.1 t	2″, 10″
	1.23 (*td*, 12.8, 4.1)		2″, 3″, 9″, 10″, 20″	1.23 (*td*, 13.0, 3.6)		2″, 10″
2″	1.70–1.57[a]	19.2 t	ND	1.70–1.57[a]	19.2 t	ND
	1.70–1.57[a]		ND	1.70–1.57[a]		ND
3″	1.80 (*td*, 12.6, 4.2)	37.8 t	1″, 4″, 5″	1.92–1.80[a]	37.8 t	1″, 2″, 4″, 5″
	1.62–1.57[a]		1″, 4″, 5″	1.60 (*td*, 12.8, 2.8)		1″, 2″, 4″, 5″
4″		47.7 s			47.7 s	
5″	2.03 (*dd*, 12.8, 2.5)	50.4 d	4″, 6″, 7″, 9″, 10″, 18″, 19″, 20″	2.04 (*dd*, 12.4, 2.7)	50.4 d	4″, 6″, 7″, 9″, 10″, 18″, 19″, 20″
6″	1.46 (*td*, 12.8, 4.2)	27.3 t	5″, 7″, 8″,10″	1.46 (*td*, 12.8, 4.3)	27.3 t	5″, 7″, 10″
	1.36 (*ddt*, 12.8, 7.5, 2.5)		5″, 7″, 8″,10″	1.37 (*ddt*, 12.8, 7.5, 2.6)		5″, 7″,10″
7″	2.34 (*ddd*, 12.8, 4.2, 2.5)	38.4 t	5″, 6″, 8″, 9″, 17″	2.35 (*ddd*, 12.8, 4.1, 2.1)	38.4 t	5″, 6″, 8″, 9″, 17″
	2.06–2.00[a]		5″, 6″, 8″, 9″, 17″	2.02 (*td*, ND, 5)		5″, 6″, 8″, 9″, 17″
8″		148.8 s			148.9 s	
9″	1.91–1.84[a]	58.1 d	8″, 11″, 12″, 17″, 20″	1.92–1.82[a]	58.0 d	8″, 11″, 17″, 20″
10″		39.6 s			39.5 s	
11″	2.39 (*ddd*, 15.2, 6.2, 1.8)	23.2 t	8″, 9″, 12″, 13″	2.38–2.32[a]	23.2 t	8″, 9″, 12″, 13″
	2.16 (*ddd*, 15.2, 10.8, 6.2)		8″, 9″, 12″, 13″	2.20 (*ddd*, 15.3, 11.0, 7.1)		8″, 9″, 12″, 13″
12″	5.57 (*t*, 6.2)	130.2 d	9″, 11″, 13″, 14″, 16″	5.54 (*t*, 6.4)	130.3 d	9″, 11″, 14″, 16″
13″		133.9 s			133.9 s	
14″	4.42 (*dd*, 10.3, 1.8)	83.4 d	7′, 12″, 13″, 15″, 16″	4.42 (*dd*, 10.3, 2.0)	83.4 d	7′, 12″, 13″, 15″, 16″
15″	1.97–1.84[a]	25.9 t	ND	1.96 (*ddt*, 13.5, 6.5, 2.0)	26.0 t	7′, 14″
16″	1.80 (*s*)	12.6 q	12″, 13″, 14″	1.80 (*s*)	12.6 q	12″, 13″, 14″
17″	4.82 (*d*, 1.3)	108.3 t	7″, 8″, 9″	4.83 (*d*, 1.2)	108.4 t	7″, 8″, 9″
	4.53 (*d*, 1.3)		7″, 8″, 9″	4.53 (*d*, 1.2)		7″, 8″, 9″
18″		179.7 s			179.7 s	
19″	1.14 (*s*)	17.1 q	3″, 4″, 5″	1.14 (*s*)	17.1 q	3″, 4″, 5″
20″	0.79 (*s*)	15.0 q	1″, 5″, 9″, 10″	0.79 (*s*)	15.0 q	1″, 5″, 9″, 10″
3-OH	10.16 (*s*)	–	2, 3, 4	10.16 (*s*)	–	2, 3, 4
5-OH	18.57 (*s*)	–	4, 5, 6, 10	18.57 (*s*)	–	4, 5, 6, 10
2′-OH	16.48 (*s*)	–	1′, 2′, 3′, 8′	16.48 (*s*)	–	1′, 2′, 3′, 8′
6′-OH	11.26 (*s*)	–	1′, 5′, 6′	11.26 (*s*)	–	1′, 5′, 6′

[a] Overlapping signals, ND: Not determined due to overlap with solvent or other signals.

Wallichin D (**4**) was obtained together with wallichin C (**3**) by HPLC processing of one of the phloroglucinol containing fractions (Rt 35 and 33 min, respectively) and was assigned the formula $C_{42}H_{54}O_{10}$ on the basis of HREIMS. This compound has the same molecular formula of **1**–**3**. The 1D NMR spectra of compound **4** were identical to those of **3**, but different relative intensities of the main peaks in their EIMS spectra indicated that they were not the same. Moreover, their specific rotation differed significantly (−66.6 and +43.1 for compounds **3** and **4**, respectively). It was reasonable to think that wallichins C (**3**) and D (**4**) were epimers at C-14″. In order to confirm this hypothesis, CD spectra were recorded for both compounds. They showed bands with opposite sign all along their spectra (Supporting Information, S23). The absolute configuration at C-14″ was established as *R* by comparison of the experimental CD spectrum of **4** with those calculated for both epimers (see Fig. 9).

2.2. In vitro nematocidal activity

Plants have been used, throughout history, for their medicinal properties. Due to the fact that *Dryopteris* ferns were employed to treat helminthiasis in traditional medicine and ethnoveterinary (Githiori et al., 2006; Murakami and Tanaka, 1988), the *in vitro* anthelmintic activity of the pure acylphloroglucinols was evaluated against the rat parasitic nematode *N. brasiliensis* L4.

All tested acylphloroglucinols showed moderate activity (Table 3), though significantly lower than the reference drug albendazole (LD_{50} = 0.3 μM), with wallichin B (**2**) being the most active (LD_{50} = 22 μM). Some qualitative structure–activity relationships could be derived from the results. Filixic acids are more active (LD_{50} = 28–37 μM) than albaspidins (LD_{50} = 106–121 μM). Concerning terpenylated phloroglucinol derivatives **1**, **2**, and **3**, the presence of a free hydroxyl group at C-4′ seems to accentuate the activity, since wallichins A (**1**) and B (**2**) are considerably more active (LD_{50} = 34 and 22 μM, respectively) than wallichin C (**3**) (LD_{50} = 118 μM).

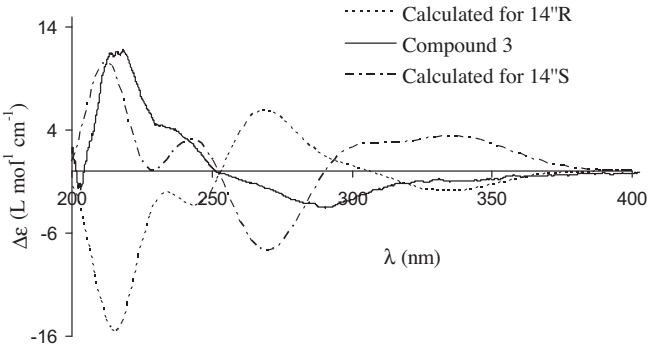

Fig. 8. Experimental CD spectrum of compound **3** (ethanol solution) compared with simulated ECD spectra of both epimers at C-14″ (14″*S* and 14″*R*).

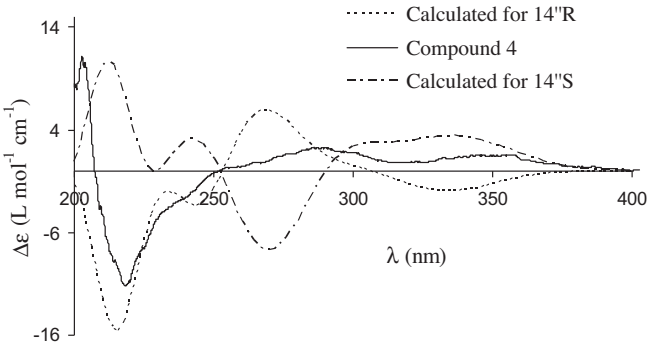

Fig. 9. Calculated ECD spectra of both isomers at C-14″ (14″*S* and 14″*R*) compared with the experimental CD spectrum of wallichin D (**4**) in ethanol solution.

Table 3
In vitro anthelmintic activity of acylphloroglucinols from the fern *D. wallichiana* against L4 larvae of *N. brasiliensis*.

Compound	LD_{50} [μM] (CI_{95})[a]	LD_{50} [ppm] (CI_{95})[a]
Wallichin A (**1**)	34 (29; 40)	24.4 (20.6; 28.9)
Wallichin B (**2**)	22 (13; 36)	15.8 (9.5; 26.2)
Wallichin C (**3**)	118 (93; 148)	84.4 (66.8; 106.6)
Albaspidin AA	106 (72; 157)	43.0 (29.1; 63.6)
Albaspidin AB	121 (96; 153)	52.2 (41.4; 66.0)
Filixic acid ABA	28 (20; 37)	16.9 (12.6; 22.8)
Filixic acid ABB	37 (21; 65)	23.5 (13.3; 41.6)
Albendazole	0.3	0.08

[a] CI_{95}: 95% confidence interval.

3. Conclusions

Terpenylated acylphloroglucinols are rare in nature and display fascinating biological activities. The present report constitutes an important contribution to the knowledge of the absolute stereochemistry of acylphloroglucinols. The determination of the absolute configuration of compounds **1**–**4** by simulation of their ECD spectra provides another example of the utility of this computational method even for complex molecules such as these. All evaluated acylphloroglucinols show moderate nematocidal activity against *N. brasiliensis*.

4. Experimental section

4.1. General experimental procedures

Optical rotations were measured on a JASCO P-1030 digital polarimeter. UV and CD spectra were recorded on a JASCO V-550 spetrophotometer and a JASCO J-810 spectropolarimeter, respectively. Infrared spectra were acquired on a Shimadzu FT/IR-8400S spectrophotometer by the diffuse reflectance method. MS analyses were conducted on a JEOL JMS AX-500 spectrometer. NMR spectra were measured at 600 MHz for ^{1}H and 150 MHz for ^{13}C on a Varian Unity 600, using acetone-d_6 as solvent and internal standard. Column chromatography (CC) was carried out over silica gel 60 (70–230 mesh, Merck), using an *n*-hexane–EtOAc gradient as mobile phase. Gel filtration chromatography was performed over Sephadex-LH 20, using a mixture of CH_2Cl_2–MeOH (1:1) as eluent. The eluate of each column was monitored by TLC on glass precoated plates F_{254} and Godin reagent was used to visualize the spots (Godin, 1954). Preparative HPLC was carried out on a Gilson equipment, using a Si gel column (*Chemcopak*; *Chemcosorb* 5 Si–U, 5 μm, 250 × 10 mm i.d.), and ultraviolet and refractive index detectors in parallel.

4.2. Plant material

D. wallichiana was collected on route 324 near Yacuchina, Tucumán Province, Argentina, in December 2008. Plant material was identified by Marcela A. Hernández and a voucher specimen (Socolsky y Bardón 14) was deposited at the Herbarium of the Fundación Miguel Lillo, Tucumán, Argentina.

4.3. Extraction and isolation

The air dried rhizomes and scales of *D. wallichiana* (138 g) were ground and extracted with Et_2O, and the extract (10.9 g) submitted to silica gel CC to yield two acylphloroglucinol-containing fractions: I (388 mg) and II (1.3 g). Fraction I was first purified by gel filtration on Sephadex LH-20 to eliminate chlorophylls, and further processed by NPHPLC (85:15 *n*-hexane–EtOAc, 1% HOAc, 3.5 mL/min) to give two fractions (A and B). Fraction A was reprocessed by HPLC (95:5 *n*-hexane–EtOAc, 1% HOAc, 4.0 mL/min) to afford compounds **3** (Rt: 33 min, 44.4 mg, yield: 0.4% of crude extract) and **4** (Rt: 35 min, 24.3 mg, 0.2% yield). NPHPLC of fraction B yielded albaspidin AA (14.7 mg, 0.1% yield), albaspidin AB (2.5 mg, 0.02% yield), filixic acid ABA (27.5 mg, 0.2% yield), and filixic acid ABB (6.4 mg, 0.06% yield). Fraction II was processed on a Sephadex LH-20 column to give chlorophyll free fraction C (878 mg). HPLC of a portion (222 mg) of fraction C (92:8 *n*-hexane–EtOAc, 1% HOAc) afforded acylphloroglucinols **1** (19.3 mg, 0.7% yield) and **2** (7.9 mg, 0.3% yield).

4.3.1. Wallichin A (**1**)

Yellow gum; $[\alpha]_D^{22.0}$ +23.6 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1}: 3219, 3082, 2741, 2660, 1693, 1639, 1614; UV (EtOH) λ_{max} ($\log\varepsilon$): 354 (4.14), 303 (4.34), 221 (4.39); for ^1H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) spectroscopic data, 2CC Table 1; CD (3.3×10^{-5} M, EtOH) λ_{max} nm ($\Delta\varepsilon$): 206 (+23.40), 228 (−12.05), 299 (+1.53), 332 (+0.72), 361 (−0.89) in Fig. 5; HREIMS, 75 eV, *m/z*: 718.3723 (calcd for $C_{42}H_{54}O_{10}$: 718.3718); EIMS *m/z* (rel int): 718 (0.2) $[M]^+$, 510 (3), 404 (3), 209 (24), 208 (100), 196 (18), 193 (50), 165 (20).

4.3.2. Wallichin B (**2**)

Reddish gum; $[\alpha]_D^{22.6}$ −27.1 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1}: 3225, 3080, 2741, 2656, 1695, 1614; UV (EtOH) λ_{max} ($\log\varepsilon$): 355 (3.94), 302 (4.12), 221 (4.16); for ^1H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) spectroscopic data 2CC Table 1; CD (6.8×10^{-5} M, EtOH) λ_{max} nm ($\Delta\varepsilon$): 208 (−27.44), 231 (+12.22), 301 (+0.96), 339 (−0.84), 366 (+0.72) in Fig. 6; HREIMS, 75 eV, *m/z*: 718.3719 (calcd for $C_{42}H_{54}O_{10}$: 718.3718); EIMS *m/z* (rel int): 718 (0.1) $[M]^+$, 510 (2), 404 (45), 209 (48), 208 (100), 196 (67), 193 (89), 165 (36).

4.3.3. Wallichin C (**3**)

Yellow gum; $[\alpha]_D^{21.8}$ −66.6 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1}: 3142, 3082, 2721, 2650, 1693, 1636, 1606; UV (EtOH) λ_{max} ($\log\varepsilon$): 356 (4.20), 304 (4.30), 218 (4.45); for ^1H NMR (600 MHz, acetone-d_6) and, ^{13}C NMR (150 MHz, acetone-d_6) spectroscopic data, 2CC in Table 2; CD (3.5×10^{-5} M, EtOH) λ_{max} nm ($\Delta\varepsilon$): 202 (−1.59), 216 (+11.60), 230 sh (+4.58), 289 (−3.51), 340 (−0.96) in Fig. 8; HREIMS, 75 eV, *m/z*: 718.3706 (calcd. for $C_{42}H_{54}O_{10}$: 718.3718); EIMS *m/z* (rel int): 718 (1) $[M]^+$, 510 (14), 209 (88), 208 (100), 193 (56), 165 (27).

4.3.4. Wallichin D (**4**)

Yellow gum; $[\alpha]_D^{21.8}$ +43.1 (*c* 1.0, $CHCl_3$); IR ν_{max}^{neat} cm^{-1}: 3136, 3080, 2719, 2652, 1693, 1636, 1606; UV (EtOH) λ_{max} ($\log\varepsilon$): 356 (4.27), 304 (4.36), 219 (4.51); for ^1H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) spectroscopic data, 2CC Table 2; CD (3.3×10^{-5} M, EtOH) λ_{max} nm ($\Delta\varepsilon$): 203 (+11.20), 218 (−11.18), 238 sh (−2.90), 287 (+2.23), 344 (+1.57) in Fig. 9; HREIMS, 75 eV, *m/z*: 718.3719 (calcd. for $C_{42}H_{54}O_{10}$: 718.3718); EIMS *m/z* (rel int): 718 (0.3) $[M]^+$, 510 (18), 209 (100), 208 (55), 193 (30), 165 (20).

4.4. Determination of the absolute configuration of wallichins A–D (**1**–**4**) at C-14″

All calculations were carried out with the Gaussian 03 program (Frisch et al., 2004). Geometries of the hypothetical isomers were first optimized using a density functional theory (DFT) method (B3LYP) with the 6–31G* basis set. PCM model was used to take into account the effect of the solvent (EtOH) on the electronic structures of the evaluated systems. Time dependent density functional theory (TD-DFT) was employed to perform the calculations for the excited states at the ground-state optimized geometries. These calculations were performed using the same method and basis set applied to the ground-state calculations to obtain the excitation energies and rotatory strengths of the lowest 50 spin-allowed excited states. ECD spectra were simulated from these data after applying the following equations:

$$\Delta\varepsilon_n = \frac{\lambda_n R_n}{22.94\sqrt{\pi}\Delta\lambda_n} \times 10^{40}$$

where $\Delta\varepsilon_n$ is the peak intensity of the n^{th} transition in L mol^{-1} cm^{-1}, λ_n is the wavelength of the mentioned transition in cm, R_n is its rotatory strength, and $\Delta\lambda_n$ is the width at 1/e of the peak maximum. This last parameter is defined as $\Delta\lambda_n = \lambda_n{}^2\Delta\nu$, with $\Delta\nu = 2500$ cm^{-1} in this particular case.

$$\Delta\varepsilon = \sum_n \Delta\varepsilon_n \exp\left[-\left(\frac{\lambda-\lambda_n}{\Delta\lambda_n}\right)^2\right]$$

4.5. Anthelmintic activity in an in vitro model of N. brasiliensis L4

The effect of pure acylphloroglucinols from *D. wallichiana* on L4 larvae of *N. brasiliensis* was evaluated as described previously (Gordon et al., 1997). Tests were carried out in tissue-culture 24-well plates. Culture medium (1.8 mL) was poured into each well, followed by 50 larvae suspended in 0.2 mL of the same culture medium. L4 larvae used in this test were recovered from male Wistar rats (60 days old) at the third day post-infection with L3 larvae of *N. brasiliensis*.

In all cases, compounds were dissolved in dimethyl sulfoxide (DMSO) and used immediately. Appropriate dilutions in DMSO were prepared for each sample to be tested in order to obtain the desired concentration after the addition of 10 μL of the solution into each well. The assay was carried out in four replicates for each concentration. The number of dead worms was determined after 5 days and corrected by controls. The recorded mortality was used to calculate LD_{50} by probit analysis performed on a software computer program. Control experiments were performed by pouring 10 μL of DMSO in the culture medium. Albendazole was used for the positive control and as reference drug (LD_{50} = 0.3 μM).

Acknowledgements

Authors thank Marcela A. Hernández for the identification of the plant material, Ms. Okamoto for the measurement of MS spectra, and Mr. Tanaka for registering 600 MHz NMR spectra. We gratefully acknowledge Dr. J. Saldaña, Universidad de la República Oriental del Uruguay, for performing the bioassay. Computer power was gently provided by the SimOne cluster at INSIBIO (UNT-CONICET) C. Socolsky thanks the Ministry of Education, Culture, Sports, Science, and Technology of Japan for a fellowship. This work was supported in part by ANPCyT, CONICET, and CIUNT, Argentina.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2012.04.017.

References

Chopra, R.N., Chopra, I.C., Handa, K.L., Kapur, L.D., 1985. Medicinal Ferns. In: Dhur, B.K. (Ed.), Indigenous drugs of India. Academic Publishers, Kolkata, pp. 649–650.

v. Euw, J., Lounasmaa, M., Reichstein, T., 1980. Chemotaxonomy in Dryopteris and related fern genera. Studia Geobot. 1, 275–311.

v. Euw, J., Reichstein, T., Widén, C.-J., 1985. The phloroglucinols of *Dryopteris aitoniana* Pichi Serm. (Dryopteridaceae, Pteridophyta). Helv. Chim. Acta 68, 1251–1275.

Fraser-Jenkins, C.R., Widén, C.-J., 1993. Phloroglucinol derivatives in *Dryopteris ardechensis* and *D. corleyi* (Pteridophyta, Dryopteridaceae) and their putative ancestors. Ann. Bot. Fennici 30, 43–51.

Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Montgomery, Jr., J.A., Vreven, T., Kudin, K.N., Burant, J.C., Millam, J.M., Iyengar, S.S., Tomasi, J., Barone, V., Mennucci, B., Cossi, M., Scalmani, G., Rega, N., Petersson, G.A., Nakatsuji, H., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Klene, M., Li, X., Knox, J.E., Hratchian, H.P., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Ayala, P.Y., Morokuma, K., Voth, G.A., Salvador, P., Dannenberg, J.J., Zakrzewski, V.G., Dapprich, S., Daniels, A.D., Strain, M.C., Farkas, O., Malick, D.K., Rabuck, A.D., Raghavachari, K., Foresman, J.B., Ortiz, J.V., Cui, Q., Baboul, A.G., Clifford, S., Cioslowski, J., Stefanov, B.B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Martin, R.L., Fox, D.J., Keith, T., Al-Laham, M.A., Peng, C.Y., Nanayakkara, A., Challacombe, M., Gill, P.M.W., Johnson, B., Chen, W., Wong, M.W., Gonzalez, C., Pople, J.A., 2004. Gaussian 03, Revision C.02. Gaussian, Inc., Wallingford, CT.

Fuchino, H., Nakamura, H., Wada, H., Hakamatsuka, T., Tanaka, N., 1997. Two new acyl-phloroglucinols from *Dryopteris atrata*. Chem. Pharm. Bull. 45, 1101–1102.

Githiori, J.B., Athanasiadou, S., Thamsborg, S.M., 2006. Use of plants in novel approaches for control of gastrointestinal helmints in livestock with emphasis on small ruminants. Vet. Parasitol. 139, 308–320.

Godin, P., 1954. A new spray reagent for paper chromatography of polyols and cetoses. Nature 174, 134.

Gordon, S., Incerti, M., Manta, E., Saldaña, J., Domínguez, L., Mariezcurrena, R., Suescun, L., 1997. Synthesis and *in vitro* anthelmintic activity against *Nippostrongylus brasiliensis* of 2-amino-delta-valerolactam derivatives. Il Farmaco 52, 603–608.

Hisada, S., Noro, Y., 1961. On the pharmacological studies of ferny drugs. VIII. Pharmaceutical studies on Japanese ferns containing phloroglucinol derivatives. (5). On the constituents of *Dryopteris* genus by paper electrophoresis. Yakugaku Zasshi 81, 1270–1279.

Ishiguro, K., Nagata, S., Fukumoto, H., Yamaki, M., Isoi, K., 1994. Phloroglucinol derivatives from *Hypericum japonicum*. Phytochemistry 35, 469–471.

Jiemchooroj, A., Norman, P., 2007. Electronic circular dichroism spectra from the complex polarization propagator. J. Chem. Phys. 126, 134102.

Kuo, Y.J., Hwang, S.Y., Wu, M.D., Liao, C.C., Liang, Y.H., Kuo, Y.H., Ho, H.O., 2008. Cytotoxic constituents from *Podocarpus fasciculus*. Chem. Pharm. Bull. 56, 585–588.

Lounasmaa, M., Karjalainen, A., Widén, C.J., Huhtikangas, A., 1972. Mass spectral studies on some naturally occurring phloroglucinol derivatives. Part III. The mass spectra of some mono- and bicyclic phloroglucinol derivatives from rhizomes of different *Dryopteris* species. Acta Chem. Scand. 26, 89–101.

Lounasmaa, M., 1978. Dérivés phloroglucinoliques des fougères du genre *Dryopteris*. Planta Med. 33, 173–176.

Lounasmaa, M., 1979. Spectrométrie de masse en ionization chimique des dérivés phloroglucinoliques des fougères du genre *Dryopteris*. Planta Med. 37, 151–155.

Minami, T., Wada, S., Tokuda, H., Tanabe, G., Muraoka, O., Tanaka, R., 2002. Potential antitumor-promoting diterpenes from the cones of *Pinus luchuensis*. J. Nat. Prod. 65, 1921–1923.

Murakami, T., Tanaka, N., 1988. Occurence, structure and taxonomic implications of fern constituents. In: Herz, W., Grisebach, H., Kirby, G.W., Tamm, C. (Eds.), Progress in the Chemistry of Organic Natural Products, 54. Springer, Vienna, p. 353.

Rocha, L., Marston, A., Potterat, O., Kaplan, M.A.C., Hostettmann, K., 1996. More phloroglucinols from *Hypericum brasiliense*. Phytochemistry 42, 185–188.

Socolsky, C., Asakawa, Y., Bardón, A., 2007. Diterpenoid glycosides from the bitter fern *Gleichenia quadripartita*. J. Nat. Prod. 70, 1837–1845.

Socolsky, C., Borkosky, S.A., Asakawa, Y., Bardón, A., 2009. Molluscicidal phloroglucinols from the fern *Elaphoglossum piloselloides*. J. Nat. Prod. 72, 787–790.

Socolsky, C., Borkosky, S.A., Hernández de Terán, M., Asakawa, Y., Bardón, A., 2010a. Phloroglucinols from the Argentine ferns *Elaphoglossum gayanum* and *E. piloselloides*. J. Nat. Prod. 73, 901–904.

Socolsky, C., Arena, M.E., Asakawa, Y., Bardón, A., 2010b. Antibacterial prenylated acylphloroglucinols from the fern *Elaphoglossum yungense*. J. Nat. Prod. 73, 1751–1755.

Socolsky, C., Borkosky, S., Bardón, A., 2011a. Structure-molluscicidal activity relationships of acylphloroglucinols from ferns. Nat. Prod. Commun. 6, 387–391.

Socolsky, C., Cartagena, E., Asakawa, Y., Bardón, A., 2011b. Acylphloroglucinols from the fern *Elaphoglossum lindbergii*. Arkivoc 2011, 450–460.

Tryon, R., Widén, C.-J., Huhtikangas, A., Lounasmaa, M., 1973. Phloroglucinol derivatives in *Dryopteris paralellogramma* and *D. patula*. Phytochemistry 12, 683–687.

Widén, C.-J., Faden, R.B., Lounasmaa, M., Vida, G., v. Euw, J., Reichstein, T., 1973. Die Phloroglucide von neun *Dryopteris*-Arten aus Kenya sowie der *D. oligodonta* (*Desv.*) Pic.-Serm. und *D.* "*dilatata*" von den Canarischen Inseln. Helv. Chim. Acta 56, 2125–2151.

Widén, C.-J., Widén, K., Vida, G., Reichstein, T., 1991. The phloroglucinols of the *Dryopteris villarii* complex and some related ferns (Pteridophyta, Dryopteridaceae). Bot. Helv. 101, 77–120.

Widén, C.-J., Äyräs, P., Reichstein, T., 1992. The phloroglucinols of *Dryopteris stenolepis* (Pteridophyta, Dryopteridaceae). Ann. Bot. Fennici 29, 41–54.

Widén, C.-J., Pyysalo, H., Reichstein, T., 1994. Fast-atom-bombardment mass spectra of phloroglucinols from *Dryopteris* ferns. Helv. Chim. Acta 77, 1985–1998.

Widén, C.-J., Fraser-Jenkins, C., Reichstein, T., Gibby, M., Sarvela, J., 1996. Phloroglucinol derivatives in *Dryopteris* sect. *Fibrillosae* and related taxa (Pteridophyta, Dryopteridaceae). Ann. Bot. Fennici 33, 69–100.

Wollenweber, E., Stevens, J.F., Ivanic, M., Deinzer, M.L., 1998. Acylphloroglucinols and flavonoid aglycones produced by external glands on the leaves of two *Dryopteris* ferns and *Currania robertiana*. Phytochemistry 48, 931–939.

Wu, X.F., Hu, Y.C., Yu, S.S., Jiang, N., Ma, J., Tan, R.X., Li, Y., Lv, H.N., Liu, J., Ma, S.G., 2011. Lysidicins F-H, three new phloroglucinols from *Lysidice rhodostegia*. Org. Lett. 12, 2390–2393.