2012

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PHYTOCHEMISTRY, OXFORD, v. 74, n. 1, pp. 166-172, FEB, 2012

Phytochemistry 74 (2012) 166–172

Contents lists available at SciVerse ScienceDirect

Phytochemistry

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

Secondary metabolites from *Spirotropis longifolia* (DC) Baill and their antifungal activity against human pathogenic fungi

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ARTICLE INFO

Article history:
Received 29 August 2011
Received in revised form 20 October 2011
Available online 1 December 2011

Keywords:
Spirotropis longifolia
Leguminosae
Prenylated pterocarpans
Antifungal
Cytotoxic
Monodominant species

ABSTRACT

A phytochemical study of the ethyl acetate extract of the roots and adventitious roots of *Spirotropis longifolia*, a monodominant tree species of the Guianan rainforest, has allowed the isolation of three compounds: 2-hydroxy-8,9-methylenedioxy-2′,2′-dimethylpyrano-[5′,6′:4,3]-6a-prenyl-[6a*S*,11a*S*]-pterocarpan (spirotropin A), 2-hydroxy-8,9-methylenedioxy-2′,2′-dimethyl-3′,4′-dihydropyrano-[5′,6′:4,3]-6a-prenyl-[6a*S*,11a*S*]-pterocarpan (spirotropin B), and 5,7-dihydroxy-6,8-diprenyl-2′′′′,2′′′′-dimethylpyrano[5′′′′,6′′′′:3′,4′]-isoflavone (spirotropone). In addition, 10 known compounds, *trans*-oxyresveratrol, *trans*-resveratrol, piceatannol, daidzein, genistein, isoprunetin, lupeol, latifolol, gnetin D and gnetin E, were also isolated. These compounds were evaluated for their antifungal activity and their cytotoxicity, and their structures were established by 1D and 2D NMR, HRMS, CD and optical rotation measurements.

1. Introduction

Spirotropis longifolia (DC) Baill. is a tree whose range extends from the state of Bolívar (Venezuela) to French Guiana. This is one of the few species to form monodominant stands (>50% of individuals) in tropical rain forests and is also known for its self-coppicing ability by the constant rejuvenation of its stems (Fonty et al., 2011). From a taxonomic point of view, *S. longifolia* is the only representative of its genus, which belongs to the tribe of the Sophoreae (Leguminosae – Papilionoideae). The phytochemistry of this genus has never been investigated before, and the genus is not described in terms of plant use or medicinal properties.

In continuation of our search for antifungal compounds from durable woods (Rodrigues et al., 2010; Royer et al., 2010), ethyl acetate extracts of roots and adventitious roots of *S. longifolia* drew our attention during a screen for antifungal activity. We therefore embarked upon studying the antifungal constituents contained in the roots and adventitious roots of this species. In this article, we describe the isolation, structure elucidation from NMR spectral data, and antifungal and cytotoxic activities of all isolated compounds.

2. Results and discussion

Roots and adventitious roots were air-dried and extracted separately with ethyl acetate. Both extracts were then subjected to silica gel column chromatography, followed by reverse phase HPLC to yield three previously unreported (1–3) and 10 known (4–13) compounds (Fig. 1). In the roots, known compounds were *trans*-resveratrol (5) (Yamada et al., 2004), piceatannol (6) (Young Han et al., 2009), daidzein (7) (Li et al., 2009), genistein (8) (Kozerski et al., 2003), isoprunetin (9) (Kulesh et al., 2008), and lupeol (10) (Fotie et al., 2006). In the adventitious roots, compounds isolated were *trans*-oxyresveratrol (4) (Kanchanapoom et al., 2002), gnetin E (11) (Boralle et al., 1993), latifolol (12) (Lins et al., 1982), and gnetin D (13) (Iliya et al., 2002). These known compounds were identified by comparison of their analytical data with those reported in the literature. Compounds **11**, **12** and **13** were isolated for the first time in the Leguminosae – Papilionoideae family.

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doi:10.1016/j.phytochem.2011.10.011

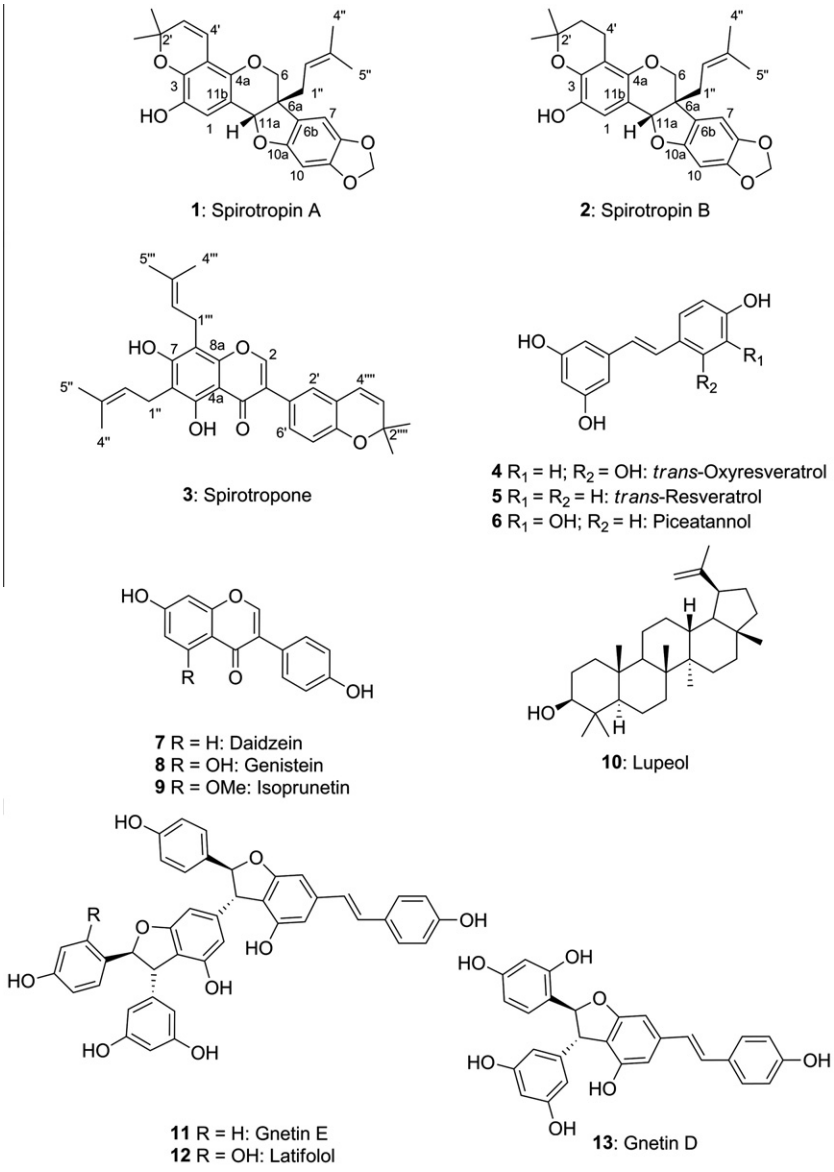

Fig. 1. Compounds isolated from *Spirotropis longifolia*.

The structure of molecules **1** and **2** were determined by ^{1}H and ^{13}C NMR spectroscopy (Table 1). Two distinct groups were observed by analysing the correlations in the COSY, HMBC and HSQC spectra: a prenyl and a dimethyl-pyrano-pterocarpan.

Compound **1** was isolated as a pale yellow solid. Its molecular formula was determined as $C_{26}H_{26}O_6$ from the HR-ESI-MS spectrum (*m*/*z*: 435.1794 $[M+H]^+$). ^{1}H NMR signal at δ_H 6.71 (1H, *s*, H-1) and carbon at δ_C 117.5 (C-1) as well as correlations of proton H-1 with C-2 (δ_C 141.0), C-3 (δ_C 142.7), C-4a (δ_C 144.6), C-11a (δ_C 83.5), and C-11b (δ_C 112.5), indicated the presence of a pentasubstituted aromatic ring. In addition, protons at δ_H 6.56 (1H, *s*, H-7) and 6.34 (1H, *s*, H-10), respectively, borne by carbon atoms at δ_C 104.8 (C-7) and 94.1 (C-10), and HMBC correlations of H-7 (δ_H 6.56) with C-8 (δ_C 149.1), C-9 (δ_C 142.9), C-10a (δ_C 155.2) and C-6a (δ_C 48.2) as well as those of H-10 (δ_H 6.34) with C-10a (δ_C 155.2), C-6b (δ_C 122.4), C-8 (δ_C 149.1) and C-9 (δ_C 142.9) together indicated the presence of a 1,2,4,5-tetrasubstituted benzene.

A methylenedioxy moiety with two non-equivalent protons at δ_H 5.87 (1H, *d*, *J* = 1.2 Hz, O–CH_2–O) and 5.83 (1H, *d*, *J* = 1.2 Hz, O–CH_2–O) was also observed in the ^{1}H NMR spectrum. HMBC correlations of this O–CH_2–O group (δ_H 5.87, δ_H 5.83) with C-8 (δ_C 149.1) and C-9 (δ_C 142.9) indicated that the oxygen atoms were linked to carbons C-8 and C-9 in the tetrasubstituted aromatic ring cited above. ^{1}H NMR signals at δ_H 6.56 (1H, *d*, *J* = 10.0 Hz, H-4′), 5.61 (1H, *d*, *J* = 10.0 Hz, H-3′) and 1.41 (6H, *s*, 2′-CH_3) combined with ^{13}C NMR signals at δ_C 117.5 (C-4′), 140.4 (C-3′), 77.3 (C-2′), and 27.7 (2′-CH_3), and their HMBC correlations indicated the presence of a 2,2-dimethyl-pyran ring. HMBC correlations of H-4′ (δ_H 6.56) with C-3 (δ_C 142.7), C-4 (δ_C 111.5) and C-4a (δ_C 144.6) as well as the chemical shift of carbon atom C-2′ at δ_C 77.3, and the molecular formula of **1** indicated that the 2,2-dimethyl-pyran ring was fused along the C-3–C-4 bond of the pentasubstituted aromatic ring. The positions of the 2,2-dimethyl-pyran ring and the methylenedioxy group were further confirmed by comparison of NMR data described for closely related 2,3-dihydroxy-8,9-methylenedioxy-pterocarpan and 6a-prenyl-pterocarpan (Miyase et al., 1999; Xiong et al., 2009). Furthermore, ^{1}H NMR spectrum of **1** (Table 1) presented a sequence of characteristic proton signals at δ_H 2.37 (2H, *m*, H-1″), 5.17 (1H, *br t*, *J* = 7.4 Hz, H-2″), 1.66 (3H, *br s*, H-4″), and 1.48 (3H, *br s*, H-5″) linked to carbon atoms at δ_C 31.6

Table 1
^{1}H and ^{13}C NMR spectroscopic data for pterocarpans **1** and **2** in CD_3OD.

Position	1				2			
	δ ^{1}H (*m*, *J* in Hz)	δ ^{13}C (δ, ppm)	COSY	HMBC (^{1}H → ^{13}C)	δ ^{1}H (*m*, *J* in Hz)	δ ^{13}C (δ, ppm)	COSY	HMBC (^{1}H → ^{13}C)
1	6.71 (1H, *s*)	117.5		C2, C3, C4a, C11a	6.70 (1H, *s*)	111.1		C2, C3, C4a, C11a
2		141.0				140.4		
3		142.7				145.6		
4		111.5				118.2		
4a		144.6				147.6		
6_{ax}	3.57 (1H, *d*, 10.7)	71.3	6-H_{eq}	C4a, C6b, C11a, C1″	3.61 (1H, *d*, 11.0)	70.9	6-H_{eq}	C4a, C11a, C1″
6_{eq}	3.99 (1H, *d*, 10.7)		6-H_{ax}	C4a, C6b, C11a, C1″	3.98 (1H, *d*, 11.0)		6-H_{ax}	C4a, C11a, C1″
6a		48.2				47.8		
6b		122.4				122.8		
7	6.56 (1H, *s*)	104.8		C8, C9, C10a, C6a	6.72 (1H, *s*)	104.7		C8, C9, C10a, C6a
8		149.1				142.2		
9		142.9				148.9		
10	6.34 (1H, *s*)	94.1		C6b, C10a, C8, C9	6.34 (1H, *s*)	93.9		C6b, C10a, C8, C9
10a		155.2				155.2		
11a	5.04 (1H, *s*)	83.5		C4a, C6, C6a, C1″, C11b	5.02 (1H, *s*)	84.1		C4a, C6, C6a, C1″, C11b
11b		112.5				114.2		
2′		77.3				71.4		
2′-CH_3	1.41 (6H, *s*)	27.7		C2′, C4′, 2′-CH_3	1.23 (6H, *s*)	28.6		C2′, C4′, 2′-CH_3
3′	5.61 (1H, *d*, 10.0)	130.4	H4′	C2′, 2′-CH_3, C4	1.66 (2H, *m*)	43.3	H3′, H4′	C2′, C4′
4′	6.56 (1H, *d*, 10.0)	117.5	H4′	C2′, C3, C3′, C4, C4a	2.69 (2H, *m*)	19.4	H3′, H4′	C2′, C3, C3′, C4, C4a,
1″	2.37 (2H, *m*)	31.6	H2″	C2″, C3″, C6b, C6a, C11a	2.45 (2H, *m*)	31.3	H1″, H2″	C2″, C3″, C6b, C6a, C11a
2″	5.17 (1H, *br t*, 7.4)	119.7	H1″, H4″, H5″	C4″, C5″	5.20 (1H, *br t*, 7.3)	119.7	H1″, H4″, H5″	C4″, C5″
3″		135.8				135.6		
4″	1.66 (3H, *br s*)	25.9	H2″	C2″, C3″, C5″	1.68 (3H, *br s*)	25.7	H2″	C2″, C3″, C5″
5″	1.48 (3H, *br s*)	24.0	H2″	C2″, C3″, C4″	1.49 (3H, *br s*)	17.7	H2″	C2″, C3″, C4″
O-CH_2-O	5.87 (1H, *d*, 1.2)	102.3		C8, C9	5.91 (1H, *d*, 1.1)	102.2		C8, C9
	5.83 (1H, *d*, 1.2)				5.88 (1H, *d*, 1.1)			

(C-1″), 119.7 (C-2″), 135.8 (C-3″), 25.9 (C-4″) and 24.0 (C-5″), respectively, attributable to a prenyl subunit in **1**. Additionally, ^{1}H NMR signals of H-6 protons at δ_H 3.57 (1H, *d*, *J* = 10.7 Hz, H-6_{ax}) and 3.99 (1H, *d*, *J* = 10.7 Hz, H-6_{eq}) borne by carbon atom at δ_C 71.3 (C-6) correlated in HMBC spectra with C-6a (δ_C 48.2), C-6b (δ_C 122.4), C-11a (δ_C 83.5), C-4a (δ_C 144.6) and C-1″ (δ_C 31.6), and a proton at δ_H 5.04 (1H, *s*, H-11a) linked to carbon atom at δ_C 83.5 (C-11a) and correlated to C-1 (δ_C 117.5), C-11b (δ_C 112.5), C-4a (δ_C 144.6), C-6 (δ_C 71.3) and C-6a (δ_C 48.2) suggested that this prenyl subunit was linked in C-6a to a pterocarpan-like compound.

The *cis* ring junction was established unambiguously by the observation of NOESY correlations between protons H-1″ and H-11a (Fig. 2). Additionally, a positive absorption band at 288.3 nm ($\Delta\varepsilon$ = 2.92) and a negative one at 327.0 nm ($\Delta\varepsilon$ = −5.42) in the CD spectrum as well as a very high positive value of the optical rotation $[\alpha]_D^{25}$ + 147.3° (AcOEt, *c* 0.164) attested of an [6a*S*, 11a*S*] absolute configuration according to literature precedent (Kiss et al., 2003). Hence, **1** has been identified as 2-hydroxy-8,9-methylenedioxy-2′,2′-dimethylpyrano-[5′,6′:4,3]-6a-prenyl-[6a*S*,11a*S*]-pterocarpan and was named spirotropin A after the name of the plant.

Compound **2** was also isolated as a pale yellow solid. Its molecular formula was determined to be $C_{26}H_{28}O_6$ by the analysis of the HR-ESI-MS spectrum (*m*/*z*: 437.1961 $[M+H]^+$). ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were very similar to those of compound **1**. It was possible to identify the penta- and tetra-substituted aromatic rings as well as the prenyl moiety in C-6a, of a pterocarpan skeleton. The only differences in the NMR signals were protons H-4′ and H-3′ and carbon atoms C-4′ and C-3′ shifted upfield from δ_H 6.56 to δ_H 2.69 for H-4′, from δ_H 5.61 to δ_H 1.66 for H-3′, from δ_C 117.5 to δ_C 19.4 for C-4′ and from δ_C 130.4 to δ_C 43.3 for C-3′. Otherwise, all other signals were nearly identical. However, we observed the presence of a new gem-dimethyl unit at δ_H 1.23 (2′-CH_3), from which it was clearly established that the 2,2-dimethyl-2*H*-pyran ring had been replaced by a 2,2-dimethyl-3,4-dihydro-2*H*-pyran ring. Again, the absolute configuration in C-6a and C-11a was ascertained as 6a(*S*), 11a(*S*) from the NOESY correlation between H-11a (δ_H 5.02) and H-1″ (δ_H 2.45), the positive sign of the optical rotation $[\alpha]_D^{25}$ + 107.6° (AcOEt, *c* 0.054) and the similarity between the CD spectra of compounds **1** and **2**. Hence, **2** was identified unambiguously as 2-hydroxy-8,9-methylenedioxy-2′,2′-dimethyl-3′,4′-dihydropyrano-[5′,6′:4,3]-6a-prenyl-[6a*S*,11a*S*]-pterocarpan and was named spirotropin B.

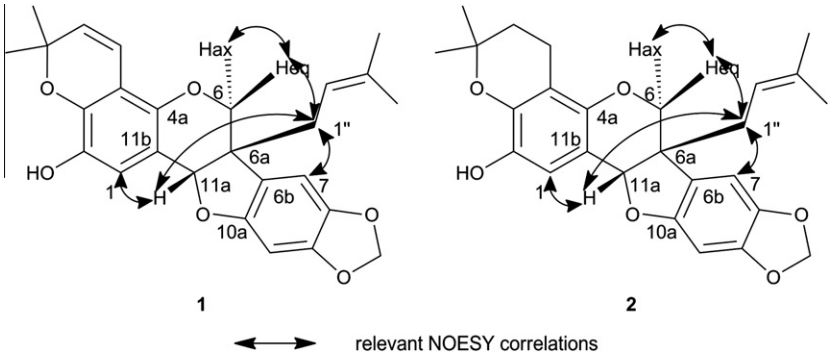

Fig. 2. NOESY correlations for compounds **1**–**3**.

Table 2
^{1}H and ^{13}C NMR spectroscopic data for spirotropone (**3**) in $CDCl_3$.

Position	**3**			
	δ ^{1}H (*m*, *J* in Hz)	δ ^{13}C (δ, ppm)	COSY	HMBC (^{1}H → ^{13}C)
2	7.90 (1H, s)	152.2		C1′, C3, C8a
3		122.9		
4		181.0		
4a		109.0		
5		157.4		
6		105.4		
7		159.5		
8		110.0		
8a		153.2		
1′		123.2		
2′	7.18 (1H, *d*, 2.2)	126.6	H6′	C3, C3′, C4′, C4′′′′, C6′
3′		121.8		
4′		152.9		
5′	6.83 (1H, *d*, 8.4)	116.5	H6′	C1′, C3′, C4′
6′	7.23 (1H, *dd*, 8.4, 2.2)	129.3	H2′, H5′	C2′, C3, C4′
1′′	3.48 (1H, *d*, 6.2)	21.5	H2′′	C2′′, C3′′, C4′′, C5, C5′′, C6, C7
2′′	5.27 (1H, *br t*, 7.1)	121.1	H1′′, H4′′, H5′′	C1′′, 4′′, C5′′
3′′		135.4		
4′′	1.77 (3H, *br s*)	25.7	H2′′	C2′′, C3′′, C5′′
5′′	1.84 (3H, *br s*)	17.7	H2′′	C2′′, C3′′, C4′′
1′′′	3.46 (1H, *d*, 6.2)	21.5	H2′′′	C2′′′, C3′′′, C4′′′, C7, C8, C8a
2′′′	5.23 (1H, *br t*, 7.3)	121.3	H1′′′, H4′′′, H5′′′	C1′′′, C4′′′, C5′′′
3′′′		134.4		
4′′′	1.74 (3H, *br s*)	25.7	H2′′′	C2′′′, C3′′′, C5′′′
5′′′	1.83 (3H, *br s*)	17.7	H2′′′	C2′′′, C3′′′, C5′′′
2′′′′		76.0		
2′′′′-CH_3	1.45 (6H, *s*)	28.0		2′′′′-CH_3, C2′′′′, C3′′′′
3′′′′	5.63 (1H, *d*, 9.8)	130.0	H4′′′′	2′′′′-CH_3, C2′′′′, C3′
4′′′′	6.35 (1H, *d*, 9.8)	122.1	H3′′′′	C2′′′′, C3′, C4′
OH-5	13.20 (1H, *s*)			C4A, C5, C6
OH-7	6.33 (1H, *s*)			C6, C7, C8

Compound **3** was isolated as a pale yellow solid. Its molecular formula was determined to be $C_{30}H_{32}O_5$ from the HR-ESI-MS spectrum (*m*/*z* 473.2323 $[M+H]^+$). First, it was established from the spectral data (Table 2) that signals at δ_H 7.18 (1H, *d*, *J* = 2.15 Hz, H-2′), 7.23 (H, *dd*, *J* = 8.4 Hz, *J* = 2.2 Hz, H-6′), and 6.83 (1H, *d*, *J* = 8.4 Hz, H-5′) indicated the presence of a 1,2,5-trisubstituted benzene, while signals at δ_H 1.45 (6H, s, 2′′′′-CH_3), 5.63 (1H, *d*, *J* = 9.8 Hz, H-3′′′′), and 6.35 (1H, *d*, *J* = 9.8 Hz, H-4′′′′) were attributable to those of a 2,2-dimethyl-2*H*-pyran ring. HMBC correlations of H-4′′′′ (δ_H 6.35) with C-3′ (δ_C 121.8) and C-4′ (δ_C 152.9) as well as the chemical shifts recorded for C-4′ (δ_C 152.9) and C-2′′′′ (δ_C 76.0) demonstrated that this ring was fused to the 1,2,5-trisubstituted benzene in positions C-3′ and C-4′. This connectivity formed a 2,2-dimethylchromene moiety substituted in C-1′ (δ_C 123.2). Additionally, an ^{1}H NMR signal at δ_H 7.90 (1H, *s*, H-2), corresponding to a proton linked to carbon at δ_C 152.2 and correlated to C-8a (δ_C 153.2) and C-3 (δ_C 122.9), was typical of an α,β-unsaturated carbonyl compound linked to an aromatic moiety. These features are common in the isoflavone family.

HMBC correlations of H-2 (δ_H 7.90) with C-1′ (δ_C 123.2) and of H-2′ (δ_H 7.18) and H-6′ (δ_H 7.23) with C-2 (δ_C 152.2) indicated that the 2,2-dimethylchromene was linked by its C-1′ to the C-3 in the α,β-unsaturated ketone. In addition, HMBC correlations of OH-5 (δ_H 13.20) with C-4a (δ_C 109.0), C-5 (δ_C 157.4) and C-6 (δ_C 105.4) and those of OH-7 (δ_H 6.33) with C-6 (δ_C 105.4), C-7 (δ_C 159.5), and C-8 (δ_C 110.0) allowed us to ascertain the presence of a hexasubstituted benzenic ring, confirming the presence of an isoflavone skeleton substituted at C-3 with a 2,2-dimethylchromene moiety. The high chemical shift of proton OH-5 (δ_H 13.20) is attributable to the proximity of the carbonyl group. It should be pointed out as well that 2 prenyl subunits at δ_H 3.48 (1H, *d*, *J* = 6.2 Hz, H-1′′), 5.27 (1H, *br t*, *J* = 7.1 Hz, H-2′′), 1.77 (3H, *br s*, H-4′′), 1.84 (3H, *br s*, H-5′′), and δ_H 3.46 (1H, *d*, *J* = 6.2 Hz, H-1′′′), 5.23 (1H, *br t*, *J* = 7.3 Hz, H-2′′′), 1.74 (3H, *br s*, H-4′′′), and 1.83 (3H, *br s*, H-5′′′) can be clearly designated in the NMR data. HMBC correlations of H-1′′ (δ_H 3.48) with C-5 (δ_C 157.4), C-6 (δ_C 105.4), and C-7 (δ_C 159.5) as well as those of H-1′′′ (δ_H 3.46) with C-7 (δ_C 159.5), C-8 (δ_C 110.0), and C-8a (δ_C 153.2) allowed us to determine their positions on the hexasubstituted benzene ring, C-6 and C-8, respectively. Based on this information and comparison with literature data described for related isoflavones (Russell et al., 1990; Xiong et al., 2009), compound **3** was designated as 5,7-dihydroxy-6,8-diprenyl-2′′′′,2′′′′-dimethylpyrano[5′′′′,6′′′′:3′,4′]-isoflavone and was named spirotropone.

All compounds isolated were tested for their antifungal activity by microdilution, most of them over 6 strains of dermatophyte

Table 3
Biological activity of isolated compounds **1** to **13**.

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	Flu*	Itra	Doc
Antifungal activity (MIC, in μg/mL)																
M. canis LMGO 25	–[a]			32 (>4[b])	–	16 (>8)			–	–	8 (3.1)		16 (>8)	2	0.5	
M. canis LMGO 22	–			–	–	–			–	–	16 (1.6)		64 (>2)	–	–	
M. gypseum LMGO 10	–			32 (>4)	–	8 (>16)		–	–	–	2 (12.5)		16 (>8)	32	0.25	
T. rubrum LMGO 08	–			16 (>8)	–	–			–	–	64 (0.4)		–	–	–	
T. rubrum LMGO 06	–			16 (>8)	–	8 (>16)		–	–	–	2 (12.5)		8 (>16)	2	0.25	
T. mentagrophytes LMGO 09	–			8 (>16)	–	64 (>2)			–	–	8 (3.1)		32 (>4)	–	–	
C. albicans LMGO 102	–			–	–	2 (>64)		16	–	–	16 (1.6)		64 (>2)	–	–	
C. albicans ATCC 10231	–			–	–	2 (>64)			–	–	16 (1.6)		2 (>64)	4	0.5	
C. parapsilosis ATCC 22019	–	–	–	–	–	4 (>32)	–	64	–	–	16 (1.6)	64 (2.0)	64 (>2)	4	0.5	
C. parapsilosis LMGO 05	–			–	–	1 (>128)			–	–	16 (1.6)		2 (>64)	4	0.5	
C. glabrata LMGO 44	–			–	–	4 (>32)		16	–	–	4 (6.3)		32 (3.9)	8	0.5	
C. tropicalis LMGO 35	–			64 (>2)	–	8 (>16)			–	–	16 (1.6)		4 (>32)	1	0.25	
C. Krusei LMGO 174	–			16 (>8)	–	8 (>16)		64	–	–	8 (3.1)		2 (>64)	32	–	
C. *gattii* LMGO L1	–			–	–	16 (>8)			–	–	8 (3.1)		64 (>2)	2	0.25	
Antiproliferative activity (IC_{50} in μg/mL)																
MRC5				> 128		> 128					25.0 ± 0.1	125.5 ± 0.5	> 128	>128	>128	< 0.25
MDA-MB-435				37.7 ± 2.4		103.3 ± 3.6					60.9 ± 0.6	64.1 ± 2.9	88.1 ± 21.8	>128	>128	< 0.25
Antiproliferative activity at 10^{-5} M (growth inhibition %)																
KB	56	0	11		0		0	0	0							99

[a] inactive (MIC > 64 g/mL, > 16 μg/mL for itraconazole).
[b] in parenthesis: selectivity index based on cytotoxicity against MRC5 cells, defined as the ratio IC_{50}/MIC; blank: not tested.
* Flu: Fluconazole, Itra: Itraconazole, Doc: Docetaxel.

fungi and 8 pathogenic yeasts. The antifungal activities were compared to those of positive standards itraconazole and fluconazole. Active compounds (MIC $\leqslant$ 64 μg/mL) were also tested towards MRC5 normal lung tissue cells and MDA-MB-435 metastatic melanoma cells to evaluate their antifungal selectivity. Meanwhile, inactive compounds were tested against KB human cervical carcinoma cells to evaluate their hypothetical cytotoxicity.

Of all the compounds tested, only **6**, **11** and **12** showed strong antifungal activity, particularly in yeast growth inhibition. Compound **6** showed the best activity against *Candida parapsilosis* LMGO 05 (MIC = 1.0 μg/mL), and compound **12** was able to inhibit *Candida albicans* ATCC 10231, *Candida parapsilosis* LMGO 05 and *Candida krusei* LMGO 174 at a concentration of 2.0 μg/mL. Moreover, compound **12** showed very high anti-yeast selectivities with index values of 62.8 when measured with MRC5 cells and 32 with MDA-MB-435 cells. In addition, the general anti-yeast activity of compound **6** was particularly interesting, with selectivities >16 with MRC5 cells and ranging from 32 to 103 with MDA-MB-435 cells.

On dermatophytes, the best antifungal activities were observed with gnetin E (11), which inhibited the growth of *Trichophyton rubrum* LMGO 06 and *Microsporum gypseum* LMGO 10 at 2.0 μg/mL, with a selectivity index of 13 in MRC5 cells and 31 in MDA-MB-435 cells. Fluconazole inhibited the growth of the latter compound at 32 μg/mL. Considering the previously unreported compounds, spirotropin A (1) presented a weak antiproliferative activity on KB cells with 56% inhibition at 10^{-5} M.

3. Concluding remarks

In conclusion, we have isolated and fully characterised three previously unreported prenylated isoflavonoids (1–3) and three compounds that had never been isolated in the Leguminosae – Papilionoideae subfamily (11–13) as well as seven others typical from this subfamily. All compounds except **10** derived from the phenylpropanoid biosynthesis pathway mixed with subsequent addition of several malonyl-CoA (Du et al., 2010) and were generally known to be phytoalexins or precursors of phytoalexins (Dixon and Ferreira, 2002; Lozovaya et al., 2007; Yamada et al., 2004). We demonstrated that several of these compounds were capable of inhibiting the growth of human pathogenic fungi and were presumably synthesised by the tree for protection against fungi-mediated degradation (Lozovaya et al., 2004; Naoumkina et al., 2007).

Torti et al. (2001) pointed out that monodominance is only allowed by an assemblage of plant traits, not any single one. Our observations may account for the natural durability and monodominance of *S. longifolia* (Fonty et al., 2011) and suggest that chemical defenses are among the plant traits allowing it. In addition, good antifungal activities and low cytotoxicities of piceatannol (6) and latifolol (12) indicated that these substances could inspire the search of new antifungal agents for the treatment of mycoses. Our study represents the first chemical description of the *Spirotropis* genus and enriches chemotaxonomic information regarding the Leguminosae – Papilionoideae subfamily.

4. Experimental

4.1. General

The optical rotations were measured on an Anton Paar MCP 300 polarimeter, and the chiroptical properties were determined with a Jasco J-810 CD spectrometer. The UV spectra were recorded with a Varian CARY 100 apparatus. The infrared spectra were recorded with a FT-IR Perkin Elmer Spectrum 100 system, and melting points were measured with a Büchi B-540 apparatus. Melting points were not corrected. Column chromatography was conducted with silica gel Merck 60 (0.063–0.2 mm). Eluting solvents were hexane and ethyl acetate (VWR extraction grade), both distilled before use, as well as methanol (VWR, analytical grade). Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian 400MR spectrometer equipped with a 5 mm Auto X PGF $^{1}H/^{15}N$-^{31}P inverse detection probe. NMR spectra were recorded at 400 MHz for ^{1}H and at 100.6 MHz for ^{13}C. Chemical shifts (δ) are in ppm downfield from tetramethylsilane, and coupling constants are in Hz. The *s* stands for singlet, *d* for doublet, *t* for triplet, and *br* for broad.

HR-ESI-MS measurements were carried out on a quadrupole-time-of-flight instrument (UltrOTOF-Q, BrukerDaltonics, Billerica, MA). Analytical HPLC was performed on a Supelco Discovery® C_{18} column (150 × 4.6 mm, 5 μm) using a Waters system equipped with a W600 pump, a W2996 photodiode array absorbance detector, and a W2420 evaporative light scattering detector. The flow rate was set to 1 mL/min using a linear gradient of water mixed with an increasing proportion of acetonitrile. Separations were performed on a Supelco Discovery® C18 column (150 × 21.2 mm, 5 μm) using a Waters system equipped with a W600 pump and a W2487 dual wavelength UV detector. The samples were injected manually through a Rheodyne injector at a flow rate of 15 mL/min, and the effluents were monitored at 214 and 254 nm. Analytical TLC plates (Si gel 60 F 254) were purchased from Fisher Scientific (France).

4.2. Plant material

Roots and adventitious roots were collected at PK18 Piste de Saint-Elie, Sinnamary, French Guiana. Identification was performed at the Cayenne herbarium, where a voucher has been deposited (CAY VE137).

4.3. Extraction and isolation

S. longifolia roots were dried, finely ground (1700 g) and extracted with ethyl acetate (3 × 3 L) at room temperature for 24 h. The organic layers were collected by filtration and combined and evaporated under reduced pressure to give the crude ethyl acetate extract (29.1 g). A portion of this extract (10.0 g) was partitioned in 10 fractions (F01–F10) by column chromatography with a polarity gradient of hexane–CH_2Cl_2–AcOEt–EtOH. Fractions were tested on *C. albicans* ATCC 10231, and the most active fraction, F-05 (1.2 g), was further separated in 15 sub-fractions F05/01 to F05/15 by column chromatography using a gradient of hexane–ethyl acetate. At this stage, lupeol (**10**, 85.0 mg, 146 ppm) was isolated in pure form. Sub-fractions F05/02 (56.0 mg) and F05/07 (53.0 mg) were purified by reverse-phase semi-preparative HPLC to give spirotropin A (**1**, 2.3 mg, 3.9 ppm) and spirotropone (**3**, 1.0 mg, 1.7 ppm). Fraction F06 was also purified by RP-HPLC to yield spirotropin B (**2**, 3.0 mg, 5.1 ppm), *trans*-resveratrol (**5**, 3.0 mg, 5.1 ppm), piceatannol (**6**, 8.0 mg, 13.7 ppm), daidzein (**7**, 1.0 mg, 1.7 ppm), genistein (**8**, 1.0 mg, 1.7 ppm), and isoprunetin (**9**, 5.5 mg, 9.4 ppm).

Adventitious roots of *S. longifolia* were dried, finely ground (85.0 g) and extracted with ethyl acetate (3 × 400 mL) at room temperature for 24 h per extraction. After filtration, the combined ethyl acetate fractions were evaporated to dryness (2.7 g). A portion of this crude extract (250 mg) was purified by RP-HPLC, allowing for the isolation of *trans*-oxyresveratrol (**4**, 1.5 mg, 0.019%), gnetin E (**11**, 1.7 mg, 0.022%), latifolol (**12**, 8.0 mg, 0.10%) and gnetin D (**13**, 1.5 mg, 0.019%).

4.4. Spirotropin A (2-hydroxy-8,9-methylenedioxy-2′,2′-dimethylpyrano-[5′,6′:4,3]-6a-prenyl-[6aS,11aS]-pterocarpan) (*1*)

Pale yellow solid. Mp 193–194 °C; $[\alpha]_D^{25}$ + 147.3° (AcOEt, *c* 0.16); CD $[\theta]_{193.8}$ 14.38, $[\theta]_{207.0}$ −31.17, $[\theta]_{223.0}$ 28.25, $[\theta]_{241.0}$ 25.17,

$[\theta]_{288.3}$ 2.92, $[\theta]_{327.0}$ −5.42 (MeCN, *c* 0.001); UV (MeCN) λ_{max} nm ($\log\varepsilon$): 298 (0.99), 210 (2.70); IR (cm^{-1}): 3484, 2979, 2924, 2888, 2866, 1740, 1643, 1572, 1473; ^1H NMR (400 MHz, CD_3OD, δ ppm): see Table 1; ^{13}C NMR (100.6 MHz, $CDCl_3$, δ ppm): see Table 1; HR-ESI-MS (positive) *m/z*: found 435.1794 $[M+H]^+$; calcd for $[C_{26}H_{27}O_6]^+$: 435.1808.

4.5. Spirotropin B (2-hydroxy-8,9-methylenedioxy-2′,2′-dimethyl-3′,4′-dihydropyrano-[5′,6′:4,3]-6a-prenyl-[6aS,11aS]-pterocarpan) (**2**)

Pale yellow solid. $[\alpha]_D^{25}$ + 108° (AcOEt, *c* 0.054); CD $[\theta]_{197.8}$ −4.34, $[\theta]_{214.5}$ 2.95, $[\theta]_{245.6}$ 1.56, $[\theta]_{241.0}$ 25.17, $[\theta]_{293.1}$ 0.53 (MeCN, *c* 0.001); UV (MeCN) λ_{max} nm ($\log\varepsilon$): 309 (0.05); ^1H NMR (400 MHz, CD_3OD, δ ppm): see Table 1; ^{13}C NMR (100.6 MHz, CD_3OD, δ ppm): see Table 1; HR-ESI-MS (positive) *m/z*: found 437.1961 $[M+H]^+$; calcd for $[C_{26}H_{29}O_6]^+$: 437.1964.

4.6. Spirotropone (5,7-dihydroxy-6,8-diprenyl-2′′′′,2′′′′-dimethylpyrano[5′′′′,6′′′′: 3′,4′]-isoflavone) (**3**)

Pale yellow solid. UV (AcOEt) λ_{max} nm ($\log\varepsilon$): 269 (1.27); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): see Table 2; ^{13}C NMR (100.6 MHz, $CDCl_3$, δ ppm): see Table 2; HR-ESI-MS (positive) *m/z*: found 473.2323 $[M+H]^+$; calcd for $[C_{30}H_{33}O_5]^+$: 473.2328.

4.7. Microorganisms and media

The species of human pathogenic microorganisms used in this study were filamentous dermatophytes *M. gypseum* LMGO 10, *Microsporum canis* (LMGO 25 and LMGO 22), *T. rubrum* (LMGO 06 and LMGO 08), and *Trichophyton mentagrophytes* LMGO 09. Yeast species were *C. albicans* (ATCC 10231 and LMGO 102), *C. parapsilosis* (ATCC 22019 and LMGO 05), *Candida glabrata* LMGO 44, *C. krusei* LMGO 174, *Candida tropicalis* LMGO 35 and *Cryptococcus gattii* LMGO L1. LMGO (Laboratório de Micologia de Goiás) strains were clinical isolates from patients at the Federal University of Goiás Hospital. Strains were maintained on potato dextrose agar. All strains were cultured onto a new agar plate and incubated at 28 °C for 2 days (yeast) or 5 days (filamentous fungi) prior to any antimicrobial test.

The strains used in cytotoxicity assays were KB cervical carcinoma cells, MRC5 normal lung tissue cells and MDA-MB-435 metastatic melanoma cells provided by the *Ciblothèque Cellulaire* of the Institute (ICSN, Gif-sur-Yvette, France).

4.8. Antifungal susceptibility assay by microdilution

The standard microdilution test described by the Clinical and Laboratory Standards Institute guidelines M27-A3 and M38-A2 was used to determine minimal inhibitory concentrations (MIC) against dermatophyte fungi and yeast (CLSI, 2008a,b; Melo e Silva et al., 2009). Pure compounds were tested at concentrations ranging from 64 to 0.125 μg/mL. Fluconazole and itraconazole (Sigma) were used as positive controls and tested at concentrations of 64–0.125 μg/mL and 16–0.031 μg/mL, respectively. The microplates were incubated at 32 °C, and the results were observed after 5 days for filamentous fungi and 2 days for yeast. The MIC values reported in Table 3 refer to the lowest concentration preventing visible growth in the wells. All assays were conducted in triplicate.

4.9. Cytotoxicity evaluation on mammalian cells

The cytotoxic effect on MRC5, KB and MDA-MB-435 cells was monitored at the *Laboratoire de Cultures Cellulaires*, ICSN, Gif-sur-Yvette, France (Tempete et al., 1995). On KB cells, the percentage of cell growth inhibition was measured at a concentration of 10^{-5} M and read after 72 h incubation. The compounds presenting antifungal activity (MIC ⩽ 64 μg/mL) were tested against MRC5 and MDA-MB-435 cells at concentrations of 128–0.25 μg/mL and read after 24 h incubation, allowing us to calculate their IC_{50} values and to evaluate their antifungal selectivity (IC_{50}/MIC). Docetaxel (Sigma) was used as a positive control and tested at concentrations of 128–0.25 μg/mL against MRC5 and MDA-MB-435 cells as well as at 10^{-5} M against KB cells.

Acknowledgments

The authors gratefully acknowledge the *Programme Amazonie* of the CNRS (France), CAPES, CNPq, FAPESP and FAPDF (Brazil) for financial support as well as J.L. Smock (IRD) for his kind help with plant collection.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2011.10.011.

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