Hypenol, a New Lignan from *Hypenia salzmannii*

by **Hellane Fabricia Sousa de Lucena**, **Sara Alves Lucena Madeiro**, **Caroline Duarte Siqueira**, **José Maria Barbosa Filho**, **Maria de Fátima Agra**, **Marcelo Sobral da Silva**, and **Josean Fechine Tavares***

Universidade Federal da Paraiba, Departamento de Ciências Farmacêuticas, Caixa Postal 5009, 58051-970, João Pessoa – PB, Brasil
(phone: +55-83-32167427; e-mail: josean@ltf.ufpb.br)

A new lignan, (−)-secoisolariciresin-9-yl 6-*O*-*p*-coumaroyl-*β*-D-glucopyranoside (**1**), named hypenol, along with eleven known compounds, *i.e.*, hyperin, rosmarinic acid, methyl rosmarinate, a mixture of the triterpenes betulinic acid, ursolic acid, and oleanolic acid, glycosylated *β*-sitosterol, a mixture of the flavanones sakuranetin and isosakuranetin, hinokinin, and *β*-peltatin A dimethyl ether were isolated from the EtOH extract of the leaves of *Hypenia salzmannii* (Benth.) Harley. The structures of the compounds were elucidated based on the analysis of spectral data, including MS, 1D- and 2D-NMR, and comparison with data in the literature.

Introduction. – The genus *Hypenia* belongs to the family Lamiaceae and consists of *ca.* 27 species with a distribution restricted to South America, especially to Venezuela, Paraguay, Bolivia, and Brazil [1]. The species *Hypenia salzmannii* is popularly known as *canela-de-urubu* and is utilized in the form of infusion and decoction of the leaves for the treatment of colds and respiratory diseases in general [2]. The medicinal use of this species prompted us to conduct a phytochemical study, in which we isolated a new lignan, (−)-secoisolariciresin-9-yl 6-*O*-*p*-coumaroyl-*β*-D-glucopyranoside (**1**), named hypenol, along with hyperin (**2**), rosmarinic acid (**3**), methyl rosmarinate (**4**), a mixture of the triterpenes betulinic acid (**5**), ursolic acid (**6**) and oleanolic acid (**7**), glycosylated *β*-sitosterol (**8**), a mixture of the flavanones sakuranetin (**9**) and isosakuranetin (**10**), hinokinin (**11**) and *β*-peltatin-A dimethyl ether (**12**).

Results and Discussion. – The EtOH extract of leaves of *Hypenia salzmannii* Benth. (Harley) was suspended in MeOH/H_2O 7:3 and successively extracted with hexane, CH_2Cl_2, and AcOEt. The AcOEt phase was submitted to a series of chromatographic separations that afforded compounds **1**–**4**, and from the CH_2Cl_2 phase compounds **5**–**12** were obtained. (−)-Secoisolariciresin-9-yl 6-*O*-*p*-coumaroyl-*β*-D-glucopyranoside (**1**) was obtained as a brown oil. HR-ESI-MS of **1** exhibited the pseudo-molecular-ion peak at *m/z* 693.2562 ($[M+Na]^+$), compatible with the molecular formula $C_{35}H_{42}O_{13}$. The IR spectrum indicated the presence of OH groups (3419 cm^{-1}), ester C=O bonds (1700 cm^{-1}), aromatic C=C bond (1604–1516 cm^{-1}), and CH_2 and CH groups (2926 cm^{-1}). The ^{13}C-NMR spectrum (Attached Proton Test) showed 31 signals attributed to two MeO, nineteen CH, five CH_2 groups, and nine quaternary C-atoms (*Table*). The signals at δ(C) 53.3 and 53.2 were attributed to two MeO groups. The signals observed between δ(C) 145.4 and 116.1 indicated aromatic

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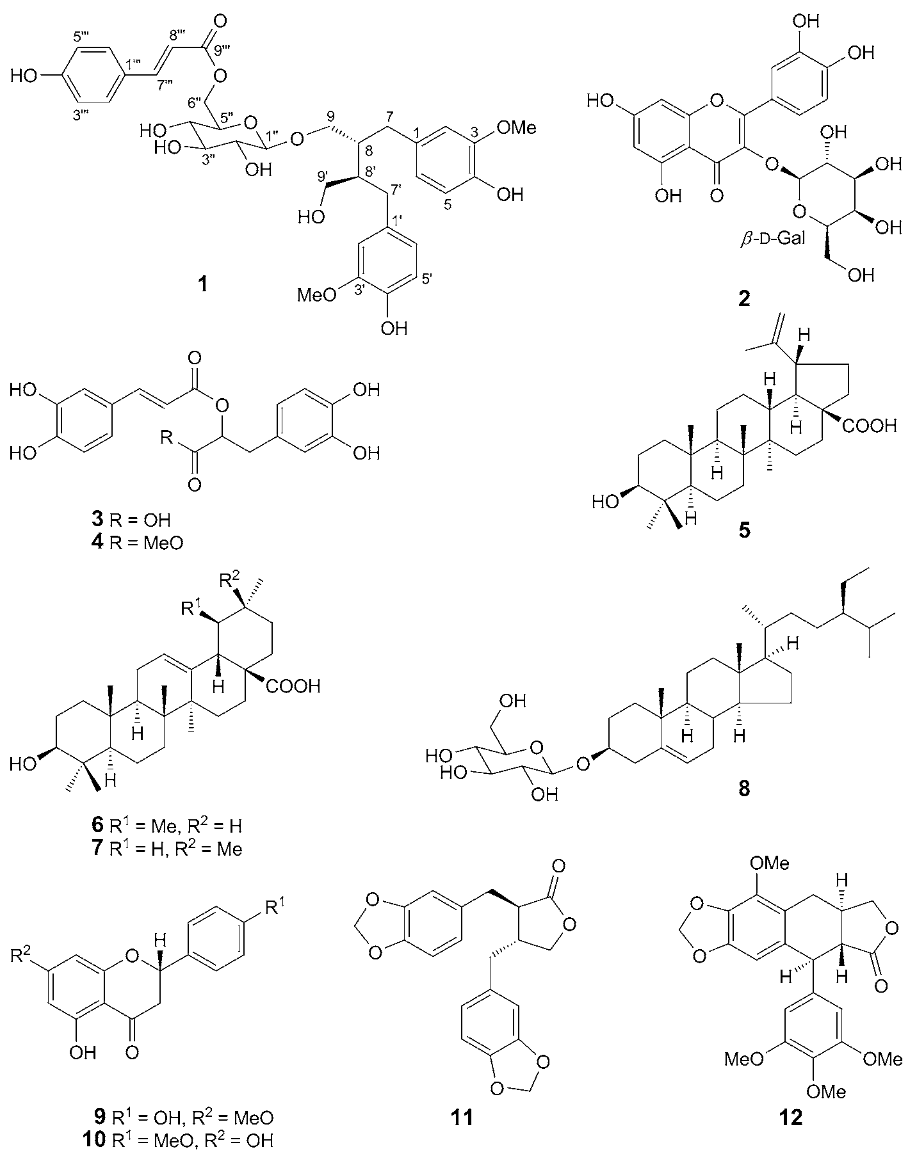

C-atoms. The signals at δ(C) 133.9, 122.8, 35.8, 44.3, 62.8, and 41.5 were typical of the lignan secoisolariciresinol structure [3][4] (*Table*). The resonances at δ(C) 104.3, 75.4, 78.0, 71.8, 75.1, and 64.6 were characteristic of the glycosidic bond [5]. The ^{1}H-NMR spectrum exhibited signals typical of the esterified *p*-coumaroyl group at δ(H) 7.38 (*d*, *J* = 8.5, 2 H) and 6.77 (*d*, *J* = 8.5, 2 H), characterizing an *AA′BB′* system, and at δ(H) 7.61 (*d*, *J* = 16.5, 1 H) and 6.32 (*d*, *J* = 16.5, 1 H), characteristic of (*E*)-coupled olefinic H-atoms [3] (*Table*). Signals for secoisolariciresinol were observed at δ(H) 6.60 (*d*,

Table. *^{1}H- and ^{13}C-NMR* (500 and 125 MHz, resp.; MeOD), *and HMBC, COSY, and NOESY Data for Compound* **1**. Arbitrary atom numbering as indicated in the *Formulae*; δ in ppm, *J* in Hz.

Position	HMQC		COSY	HMBC		NOESY
	δ(H)	δ(C)		(^2J)	(^3J)	
1	–	133.9			8	
2	6.60 (*d*, *J* = 1.5)	113.4			7	
3	–	148.8			Me	
4	–	145.4				
5	6.67 (*d*, *J* = 8.5)	115.7				
6	6.53 (*dd*, *J* = 6.0, 2.0)	122.8			7	
7	2.72 (*dd*, *J* = 14.0, 7.5) 2.63 (*dd*, *J* = 14.0, 7.0)	35.8	8		2	
8	2.00 (*m*)	41.5	7, 9	9		
9	3.83 (*m*), 3.55 (*m*)	70.1	8	8	2″, 7	2″
1′	–	133.9			8′	
2′	6.60 (*d*, *J* = 1.5)	113.6			7′	
3′	–	148.8			Me	
4′	–	145.4				
5′	6.67 (*d*, *J* = 8.5)	115.7				
6′	6.53 (*dd*, *J* = 6.0, 2.0)	122.8			7′	
7′	2.72 (*dd*, *J* = 14.0, 7.5), 2.63 (*dd*, *J* = 14.0 7.0)	35.8	8′			
8′	1.92 (*m*)	44.3	7′, 9′	9′	7	
9′	3.63 (*m*), 3.55 (*m*)	62.8	8′	8′	8, 7′	
1″	4.22 (*d*, *J* = 7.5)	104.3	2″			9
2″	3.25 (*m*)	75.1	2″			
3″	3.80 – 3.32 (*m*)	78.0				
4″	3.80 – 3.32 (*m*)	71.8				
5″	3.55 (*m*)	75.4	6″			
6″	4.49 (*dd*, *J* = 12.0, 2.0), 4.35 (*dd*, *J* = 12.0, 6.0)	64.6	5″			
1‴	–	127.1				
2‴	7.38 (*d*, *J* = 8.5)	131.2				8‴, 5‴
3‴	6.77 (*d*, *J* = 8.5)	116.8				6‴
4‴	–	161.2				
5‴	6.77 (*d*, *J* = 8.5)	116.8				2‴
6‴	7.38 (*d*, *J* = 8.5)	131.2				8‴
7‴	7.61 (*d*, *J* = 16.5)	146.8				2‴
8‴	6.32 (*d*, *J* = 15.5)	114.7				2‴, 6‴
9‴	–	169.1			6″	

J = 1.5, H–C(2,2′)); 3.55 (*m*, CH$_2$(9,9′)); 2.00 (*m*, H–C(8)); 1.92 (*m*, H–C(8′)); 6.67 (*d*, *J* = 8.5, H–C(5)); 2.72 (*dd*, *J* = 14.0, 7.5, 2 H of CH$_2$(7,7′)), 2.63 (*dd*, *J* = 14.0, 7.0, 2 H of CH$_2$(7,7′)); 6.53 (*dd*, *J* = 6.0, 2.0, 2 H for H–C(6,6′)) [4][6]. The signals at δ(C) 41.5 (C(8)) and 44.3 (C(8′)), compared with literature values [7], corroborated the *trans* configuration of the substituents at these C-atoms. Signals at δ(H) 4.49 – 3.32 were typical of glucose [5]. The HMBC ^3J between δ(H) 4.22 (*d*) and δ(C) 70.1, (C(9) indicated a bond between the anomeric C-atom of glucose, C(1″), and C(9) of the lignin (*Fig.*). The bond between the CH$_2$ C-atom of glucose C(6″) and the C=O C-atom, C(9‴), of the *p*-coumaroyl group was determined by means of the HMBC spectrum based on the correlation ^3J of δ(H) 4.49 (*dd*) and 4.35 (*dd*) with δ(C) 169.1

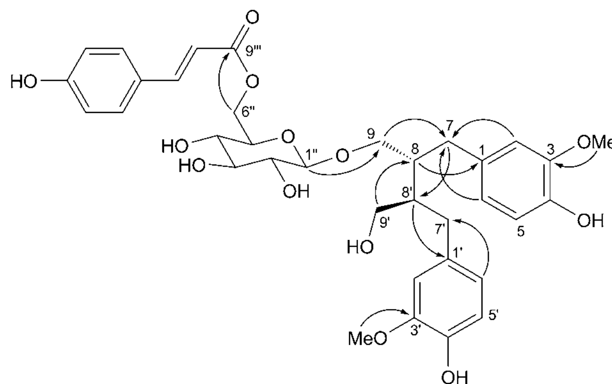

Figure. *Key HMBCs* (H → C) *of compound* **1**

(C(9''')). The NOESY spectrum showed a correlation between δ(H) 4.22 (H–C(1'')) and δ(H) 3.55 (H–C(9)).The *doublet* at δ(H) 4.22 (C(1'')) with $J = 7.5$ indicated β-anomeric configuration for the aglycone [5]. The known compounds were identified as hyperin (**2**) [8], rosmarinic acid (**3**) [9], methyl rosmarinate (**4**) [10], betulinic acid (**5**), ursolic acid (**6**) and oleanoic acid (**7**) [11], glycosylated β-sitosterol (**8**) [12], sakuranetin (**9**) and isosakuranetin (**10**) [13], hinokinin (**11**) [14], and β-peltatin-A dimethyl ether (**12**) [15][16], based on comparison of the physical and spectral data with those in the literature.

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Experimental Part

General. Column chromatography (CC): silica gel *7734* (SiO_2; 0.063 – 0.200 mm, *E. Merck*, D-Darmstadt); *Sephadex LH-20®*, *Amersham Biosciences.* TLC: SiO_2 *60* F_{254} plates (*E. Merck*). M.p.: *MQAPF 302 – Microquimica.* IR Spectra: *BOMEM-MB 100* spectrometer; in KBr; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Varian NMR System* 500 MHz; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Bruker*, *Microtof II Amazon* mass spectrometer; in *m/z*.

Plant Material. The leaves of *Hypenia salzmanni* (Lamiaceae) were collected in the municipality of Matureia, in the State of Paraíba, Brazil, and identified by *M. F. A.*, Universidade Federal da Paraíba, João Pessoa, PB, and a dried specimen of this species has been deposited with the Herbário Lauro Pires Xavier (JPB)/CCEN-UFPB (Registration of species: AGRA *et al.* 7848).

Extraction and Isolation. The plant material was dried in a circulating air oven at 35°, triturated, and extracted with 95% EtOH for 72 h. The extract was concentrated, and the residue (168.25 g) was suspended in MeOH/H_2O 7 : 3 and sucessively extracted with hexane (1.5 g), CH_2Cl_2 (11.0 g), and AcOEt (7.0 g). The AcOEt subfraction later showed the formation of a precipitate, which was saved. The CH_2Cl_2-soluble subfraction (10 g) was submitted to CC (SiO_2; elution with increasing polarity: hexane, hexane/CH_2Cl_2, CH_2Cl_2/AcOEt, AcOEt/MeOH). From *Frs. 16 – 18* (obtained with CH_2Cl_2/AcOEt 10 : 90) a mixture of compounds **5** – **7** (12.0 mg), was obtained and *Frs. 23 – 31* (obtained with AcOEt/MeOH 80 : 20) afforded compound **8** (28.0 mg). *Frs. 1 – 4* (11.0 mg; obtained with hexane/CH_2Cl_2 50 : 50) were submitted to TLC (hexane/AcOEt 70 : 30) to give compounds **11** (3.8 mg) and **12** (2.5 mg). *Frs. 5 – 10* (595.0 mg; obtained with CH_2Cl_2/AcOEt 70 : 30) were submitted to CC (SiO_2; hexane/AcOEt 50 : 50) to provide a mixture of compounds **9** and **10** (25 mg). The AcOEt-soluble subfraction (5.0 g) was

submitted to CC (*Sephadex LH-20*; MeOH): *Frs. 10–17* were submitted to CC (SiO_2; elution with increasing polarity: CH_2Cl_2/AcOEt, AcOEt/MeOH). *Frs. 9–12* and *30* furnished compounds **4** (8.5 mg) and **3** (5.0 mg), resp. *Frs. 36–40* were submitted to CC (*Sephadex LH-20*; MeOH), and *Fr. 1* gave compound **1** (11.0 mg). The precipitate from the AcOEt-soluble subfraction was submitted to CC (*Sephadex LH-20*; MeOH) and *Fr. 4* yielded compound **2** (10.0 mg).

*(–)-Secoisolariciresin-9-yl 6-*O*-*p*-Coumaroyl-β-*D*-glucopyranoside (=(2*R*,3*R*)-4-Hydroxy-2,3-bis[(4-hydroxy-3-methoxyphenyl)methyl]butyl 6-*O*-[(2*E*)-3-(4-Hydroxyphenyl)-1-oxo-2-propen-1-yl]-β-*D*-glucopyranoside*; *Hypenol*; **1**): Dark-brown oil. $[\alpha]_D^{20} = -1.2$ ($c = 0.001$, MeOH). IR (KBr): 3419, 2926, 1700, 1604–1516. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS (pos.): 693.2562 ($[M+Na]^+$, $C_{35}H_{42}NaO_{13}^+$; calc. 693.2517).

REFERENCES

[1] J. G. Silva, M. T. Faria, E. R. Oliveira, M. H. Rezende, D. G. Ribeiro, H. D. Ferreira, S. C. Santos, J. C. Seraphin, P. H. Ferri, *J. Braz. Chem. Soc.* **2011**, *22*, 955.

[2] M. F. Agra, G. S. Baracho, K. Nurit, I. J. L. D. Basílio, V. P. M. Coelho, *J. Ethnopharmacol.* **2007**, *111*, 383.

[3] Y.-H. Wang, Z.-K. Zhang, H.-P. He, S. Gao, N.-C. Kong, M. Ding, X.-J. Hao, *Acta Bot. Yunnan.* **2006**, *28*, 433.

[4] H. B. Park, K. H. Lee, K. H. Kim, I. K. Lee, H. J. Noh, S. U. Choi, K. R. Lee, *Nat. Prod. Sci.* **2009**, *15*, 17.

[5] V. C. Silva, G. H. Silva, V. S. Bolzani, M. N. Lopes, *Eclética Quím.* **2006**, *31(4)*, 55.

[6] V. B. Lima, P.h.D. Thesis, Instituto de Química-UNICAMP, 2005.

[7] H. Shibuya, Y. Takeda, R.-s. Zhang, A. Tanitame, Y.-L. Tsai, I. Kitagawa, *Chem. Pharm. Bull.* **1992**, *40*, 2639.

[8] Z. Güvenalp, L. Ö. Demirezer, *Turk. J. Chem.* **2005**, *29*, 163.

[9] U. Özgen, A. Mavi, Z. Terzi, C. Kazaz, A. Asçi, Y. Kaya, H. Seçen, *Rec. Nat. Prod.* **2011**, *5*, 12.

[10] E. R. Woo, M. S. Piao, *Arch. Pharm. Res.* **2004**, *27*, 173.

[11] D. Q. Falcão, F. S. Menezes, *Braz. J. Pharmacogn*. **2003**, *84*, 69.

[12] F. A. Medeiros, Ph.D. Thesis, Universidade Federal da Paraíba, 2008.

[13] J. M. J. Vasconcelos, A. M. S. Silva, J. A. S. Cavaleiro, *Phytochemistry* **1998**, *49*, 1421.

[14] V. C. França, K. V. M. Vieira, E. O Lima, J. M. Barbosa-Filho, E. V, L. Cunha, M. S. Silva, *Braz. J. Pharmacogn*. **2005**, *15*, 326.

[15] J. P. David, E. F. Silva, D. L. Moura, M. L. S. Guedes, R. J. Assunção, J. M. David, *Quím. Nova* **2001**, *24*, 730.

[16] A. S. Feliciano, M. Medarde, J. L. Lopez, P. Puebla, J. M. M. Corral, A. F. Barrer, *Phytochemistry* **1989**, *28*, 2863.

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