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1. Subject and source

Lycopodium cernuum L. (Lycopodiaceae), a species which is widely distributed in the tropical regions of the world including Central America, South America, Asia and Africa (Stehlé and Stehlé, 1962), was collected from Viard Petit Bourg (Guadeloupe) in August 2009, and identified by Dr. Henry Joseph. A voucher specimen (n° 10092) is deposited in the Herbarium of the National Institute of Agronomic Research (INRA-CRAAG), Guadeloupe, France.

2. Previous works

Previous phytochemical investigations on the genus *Lycopodium* (Lycopodiaceae) have resulted in the isolation of a wide variety of products, including quinolizidine, pyridine and α-pyridone alkaloids. The different alkaloids are generally designed as *Lycopodium* alkaloids (Ma and Gang, 2004) and possess interesting biological activities such as cholinesterase inhibition. Moreover, flavonoids and phenolic acids have been studied as chemotaxonomic markers in the *Lycopodium* genus (Markham and Moore, 1980; Pederson and Ollgaard, 1982). The species *L. cernuum* L. has been reported to contain cermizines A-D, cernuine, lycocernuine, cernuine *N*-oxide and lycocernuine *N*-oxide (Morita et al., 2004). Lycopodine, lycodine, lycodoline, lycopladine B, huperzines A, B and E and huperzinine have been detected in *Lycopodiella cernua* (L.) Pic. Serm. (a synonym of

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L. cernuum L.) by UHPLC/ESI-TOF-MS (Ho et al., 2009). Serratene triterpenes (lycocernuic acids A–E, lycocernuic ketones A–C, serrat-14-en-3β, 21β-diol and serrat-14-en-3β, 21α-diol) and apigenin-4′-*O*-(2″,6″-di-*O*-coumaroyl)-β-D-glucopyranoside were isolated from an ethanol extract of *L. cernuum* (Zhang et al., 2002). In several specimens of *L. cernuum*, trace amounts of esters of quinic acid, such as chlorogenic acid, were detected by electron paramagnetic resonance (EPR) (Pederson and Ollgaard, 1982). Caffeic acid was also found to be present in low amount in two specimens of *L. cernuum* (Pederson and Ollgaard, 1982).

3. Present study

The aerial parts of *L. cernuum* (500 g) were successively extracted with petroleum ether, CH_2Cl_2 and MeOH. The MeOH extract (93.1 g) was then partitioned between EtOAc (LC1) and 10% acetic acid (LC2).

LC2 were adjusted at pH10 with NaOH and were partitioned between $CHCl_3$ and H_2O. $CHCl_3$-soluble materials (383 mg) were fractionated on silica gel column (CH_2Cl_2/MeOH: 98:2–50:50 then CH_2Cl_2/MeOH + 8% NH_4OH (28%) 50:50–0:100, v/v) to afford 12 fractions. Fraction 5 consists of pure cernuine **1** (42.1 mg) (Morita et al., 2004). Fraction 6 (95.6 mg) was fractionated by silica gel column chromatography (CH_2Cl_2/MeOH 97:3–70:30, v/v) to give 7 fractions (6A–6G). Fractions 6E and 6F consisted of pure lycocernuine **2** (20.3 mg + 10.0 mg) (Morita et al., 2004). Fraction 6B (23.8 mg) was purified on preparative HPLC (column Modulo-Cart Strategy 5 RP, 250 × 10 mm, eluted with MeOH/H_2O + 0.02% NH_4OH (28%) 68:32–100:0 in 38 min) to afford compound **3** (6B3, 2.0 mg). Fraction 9 (18.8 mg) was subjected to Sephadex LH-20 gel (CH_2Cl_2/MeOH 100:0–90:10) and gave cernuine *N*-oxide **4** (1.9 mg) (Morita et al., 2004).

LC2 (18.7 g) was divided between dichloromethane and water. During this step a precipitate was formed (11.1 g) and was fractionated on silica gel column (CH_2Cl_2/MeOH 100:0–0:100, v/v) to give 16 fractions. Fraction 6 (89.1 mg) was purified on Sephadex LH-20 gel, followed by preparative HPLC (MeOH/H_2O 40/60) to yield hydroquinone **5** (1.3 mg) (Sharma et al., 1998). Purification of Fraction 7 (1.5 g) on Sephadex LH-20 gel gave apigenin-4′-*O*-(2″,6″-di-*O*-(*E*)-coumaroyl)-β-D-glucopyranoside **6** (117.9 mg) (Zhang et al., 2002) and apigenin-7-*O*-(2″,6″-di-*O*-(*E*)-coumaroyl)-β-D-glucopyranoside **7** (114.8 mg) (Itokawa et al., 1981). Water-soluble materials (900 mg) were submitted to silica gel column chromatography (CH_2Cl_2/MeOH 90:10–50:50) to yield 9 fractions. Fraction 8 was purified on Sephadex LH-20 gel to give 1-*O*-(*E*)-caffeoyl-β-D-glucopyranoside **8** (62.7 mg) (Braham et al., 2005). Known compounds were identified through UV, MS, NMR spectroscopy and by comparison with published data.

Compound **3** had the molecular formula $C_{16}H_{27}N_2O_2$ by HRMS (*m/z* 279.2065 $[M + Na]^+$, calculated for 279.2067). ^1H, ^{13}C and 2D NMR data (Table 1) implied the presence of a cernuane-type skeleton with a fused tetracyclic ring system containing two nitrogen atoms. Detailed NMR analyses and comparison with those of cernuine indicated that **3** had a closely related structure (Morita et al., 2004). The main difference with cernuine was the presence of a hydroxylated carbon in compound **3** (δ_C 69.15, δ_H 3.99). 2D NMR data made it possible to situate this hydroxylation on position 2 (Table 1). This data led to the conclusion that **1** can be identified as a new compound, 2-hydroxycernuine. Although NOE experiments made it possible to confirm that compound **3** had altogether the same configuration as that of cernuine, i.e. H-5α, H-7α, H-9β and H-13β, it was

Table 1
1D and 2D spectroscopic NMR data of 2-hydroxycernuine **3** in MeOD.

	δ^1H (multiplicity, *J* in Hertz)	COSY (H → H)	$\delta^{13}C$	HMBC (H → C)	NOESY
1	–	–	171.3	–	
2	3.99 t (5.3)	3	69.1	1, 3, 4	
3	1.86 m (2H)		27.6	1, 2, 4, 5	
4b	1.92–1.93 m (1H)		25.7	2, 3, 5, 6	
4a	1.79–1.80 m (1H)			2, 3, 5, 6	
5	3.68 m (1H)	6a, 1.67 (6b or 8b), 1.81 (4a), 1.92 (4b)	51.6		7
6a	1.31 brd (1H, 12.2)	1.67 (6b or 8b)	41.1	4, 5, 7, 8	
6b	1.67 ddd (1H, 2.7–3.0–13.0)				
7	3.27 t (1H, 10.5)	8a, 6a, 1.67 (6b or 8b)	47.9		5
8a	0.84 (1H)	1.67 (6b or 8b)	42.3	6, 7, 14, 15, 16	
8b	1.66–1.67 (1H)				
9	5.41 dd (1H, 2.7–12.1)	10a, 10b	68.8	5, 7	13
10a	1.19 brd (1H, 14.5)	10b	21.7		
10b	2.13 dd (1H, 4.0–12.9)	11b		9, 11	5, 7
11a	1.66 m (1H)		25.5	10, 12	
11b	1.95 m (1H)	1.67 (6b or 8b)			
12a	1.13 brd (1H, 14.5)	12b	23.5		
12b	1.89 m (1H)	12a		13	
13	3.09 brd (1H, 12.5)	12a, 14a, 14b, 12b	59.2		9
14a	1.41 dd (1H, 5.2–13.2)	14b	39.8	8, 12, 13, 15, 16	
14b	1.59 brd (1H, 13.2)	14a			
15	1.78 m (1H)	16	26.3		
16	0.88 d (3H, 6.5)		22.5	8, 14, 15	

Data acquired at 500 MHz for ^1H and 125 MHz for ^{13}C at 25 °C. TMS was used as internal reference; br = broad, d = doublet; dd = doublet of doublet; m = multiplet, s = singlet; t = triplet. Only relevant NOE correlations are given.

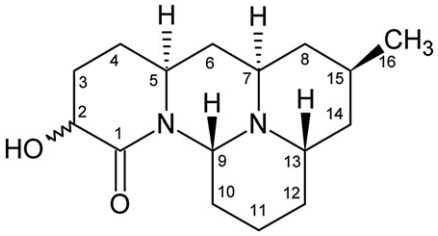

Fig. 1. Structure of 2-hydroxycernuine.

not possible to establish the orientation of CH_3-16, nor of OH-2 by means of NMR. A β-orientation was indicated in the figure for CH_3-16, as it is usually described for natural cernuane alkaloids. The bond between C-2 and the hydroxy group was left as undefined (Fig. 1).

Compound **3** (2-hydroxycernuine): white powder, $[\alpha]_D^{20} - 22^\circ$ (c 0.5, MeOH); IR (KBr) ν_{max}: 3310, 2921, 2862, 1635, 1438, 1415, 1226, 1099, 854 cm^{-1}. NMR: see Table 1. HR-ESIMS *m/z*: 279.2065 $[M + Na]^+$ (calcd. for $C_{16}H_{27}N_2O_2$, 279.2067).

4. Chemotaxonomic significance

The phenolic compounds that have been isolated from *L. cernuum* are in accordance with those usually reported in the Lycopodiaceae (Pederson and Ollgaard, 1982; Richardson, 1989). Nevertheless this is the first isolation of apigenin-7-*O*-(2″,6″-di-*O*-*p*-coumaroyl)-β-D-glucopyranoside and 1-*O*-caffeoyl-β-D-glucopyranoside in the genus *Lycopodium*.

Ma and Gang (2004) suggested a classification of *Lycopodium* alkaloids into three main classes (lycopodine, lycodine and fawcettimine classes) and a miscellaneous group to which the cernuane-type alkaloids belong.

Only the following species of *Lycopodium* contain cernuane-type alkaloids: *Lycopodium australianum* Herter, *Lycopodium carolinianum* L., *L. cernuum* L., *Lycopodium inundatum* L. and *Lycopodium laterale* R. Br. (Braekman et al., 1974, 1980; Ma and Gang, 2004). The diversity so far observed in this class is insufficient for use as a chemotaxonomic tool in order to distinguish the different species from each other. The discovery of new cernuane alkaloids, such as **3**, may provide useful insights into the taxonomy of these species.

L. cernuum L. is taxonomically ambiguous. Several species have been suggested as synonyms, such as *Lycopodiella cernua* (L.) Pic. Serm. and *Palhinhaea cernua* (L.) Franco & Vasc. (Zhao et al., 2010). Chemotaxonomic observation favours their separation into three different species. *L. cernuum* is characterised by cernuane-type alkaloids, as shown in this work and in previous ones (Ma and Gang, 2004). The main alkaloids in *L. cernua* (lycopodine and lycodoline) belong to the lycopodine class (Ho et al., 2009). Although cernuine has been detected in *P. cernua* by thin-layer chromatography (Ma et al., 1998), this was not confirmed by subsequent, more detailed investigations. Jiao et al. (2006) did not find any member of the cernuane-type nor lycopodine-class alkaloids, and Zhao et al. (2010) only described the original alkaloid palhinine A.

Thus, the three species may be classified on the basis of their content of lycopodine- or cernuine-derived alkaloid content.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bse.2012.07.026.

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