Chemical Constituents from the Roots of *Furcraea bedinghausii* Koch

Rémy B. Teponno^{1,*}, Beaudelaire K. Ponou^{1,2}, Dennis Fiorini²,
Luciano Barboni², Léon A. Tapondjou¹

¹Laboratory of Environmental and Applied Chemistry, Department of Chemistry, Faculty of Science,
University of Dschang, PO Box 183, Dschang, Cameroon

²School of Science and Technology, Chemistry Division, University of Camerino, Via S. Agostino 1,
I-62032 Camerino, Italy

*E-mail address: rteponno@yahoo.fr

ABSTRACT

Phytochemical investigation of the roots of *Furcraea bedinghausii* Koch. Led to the isolation of a mixture of two new homoisoflavones, 5,7-dihydroxy-3-(3,4-methylenedioxybenzyl)-chromone (4a) and 5,7-dihydroxy-3-(4-methoxybenzyl)-chromone (4b), together with the known β -sitosterol (1), 7,4'-dihydroxyhomoisoflavane (2), dihydrobonducellin (3), kaempferol (5), 5,7-dihydroxy-3-(4-hydroxybenzyl)-chromone (6), 1-linoleylglycerol (7), 6'-linoleyl-3-*O*- β -D-glucopyranosyl- β -sitosterol (8), *trans*-3,3',5,5'-tetrahydroxy-4'-methoxystilbene (9), yuccaol C (10), yuccaol D (11), 3-*O*- β -D-glucopyranosyl- β -sitosterol (12), 4-[6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)- β -D-glucopyranosyloxy]-3-methoxybenzoic acid (13) and two pairs of steroidal saponins: (25*R*)-2 α -3 β -dihydroxy-5 α -spirostan-12-one 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*-[β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-galactopyranoside (14a) and (25*R*)-2 α -3 β -dihydroxy-5 α -spirost-9-en-12-one 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*-[β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-galactopyranoside (14b), (25*R*)-3 β -hydroxy-5 α -spirostan-12-one 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*-[β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-galactopyranoside (15a) and (25*R*)-3 β -hydroxy-5 α -spirost-9-en-12-one 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*-[β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-galactopyranoside (15b). Their structures were elucidated by interpretation of spectral data and by comparison with literature.

Keywords: *Furcraea bedinghausii*; Agavaceae; flavonoids; Phenolic compounds; Steroidal saponins; Chemotaxonomy

1. INTRODUCTION

Furcraea bedinghausii K. Koch (Syn.: *Yucca pringlei* Greenm) belongs to the Agavaceae family which has more than 580 species widely distributed in tropic and subtropic dry climate regions [1,2]. The genus *Furcraea* has about 20 species, and is mainly cultivated as ornamental plants. Nevertheless, leaves of some plants of this genus have been used to treat ulcers, venereal diseases, rheumatism and as diuretic. They are also used as a source of fiber and to reduce swelling [3]. To the best of our knowledge, no phytochemical work is reported on *Furcraea bedinghausii*. However, phytochemical investigations on plants of the genera

Furcraea and *Yucca* afforded steroidal saponins [1,3,4] and phenolic compounds [5,6]. In this paper, the chemical survey of secondary metabolites from the roots of *Furcraea bedinghausii* was achieved, leading to the isolation and structure elucidation of several compounds including an inseparable mixture of two new homoisoflavones.

2. EXPERIMENTAL

2. 1. Plant material

The roots of *Furcraea bedinghausii* were collected in Dschang (West Region of Cameroon) in October 2009 and was identified at the Cameroon National Herbarium, Yaoundé, where a voucher specimen (Ref: HCN 25568) has been deposited.

2. 2. Instrumental techniques

IR spectra were measured as a film on a KBr pellet using a FTIR-8400S Shimadzu spectrometer. ESI mass spectra were carried out on an Agilent Technologies LC/MSD Trap SL (G2445D SL). ¹H-NMR, ¹³C-NMR, COSY, HSQC and HMBC spectra were performed in deuterated MeOH and CHCl₃ on a varian Mercury plus Spectrometer (400 MHz for ¹H and 100 MHz for ¹³C). All chemical shifts (δ) are given in ppm units with reference to tetramethylsilane (TMS) as internal standard and the coupling constants (J) are in Hz. Column chromatography was performed using Sephadex LH-20 and silica gel 60 merck (0.040-0.063 mm). TLC was carried out on precoated Kiesel 60 F₂₅₄ (Merck) plates developed with Hexane-EtOAc mixtures, EtOAc-MeOH-H₂O (80-20-10, 85-15-5, 90-10-5, 95-5-2, 97-3-1) and with EtOAc-MeOH 98-2. TLC plates were visualised under UV light (254 and 365 nm) or by spraying with 50 % aqueous H₂SO₄ and heating for 10 min at 110 °C.

2. 3. Extraction and isolation

The dried and pulverized roots of *Furcraea bedinghausii* (3.5 Kg) were extracted with methanol (2 X 10 L). The filtrate obtained was concentrated under reduced pressure to yield a dark residue (450 g). Part of this extract (430 g) was suspended in water (300 mL) and extracted with EtOAc and *n*-butanol, yielding after evaporation 24.6 g and 55 g of dried extracts, respectively. A portion of the EtOAc extract (21.6 g) was subjected to column chromatography over silica gel eluted with *n*-hexane-EtOAc and EtOAc-MeOH with increasing polarity, yielding seven main fractions (A-G). Recrystallization and filtration of fraction B (2.53 g) yielded compound **1** (250 mg) and the filtrate obtained was chromatographed on Sephadex LH-20 column eluted with CH₂Cl₂-MeOH (1:1) to afford compound **2** (15 mg) and a subfraction.

The above subfraction was repeatedly purified by silica gel column chromatography eluted with *n*-hexane-EtOAc 7:3 to yield compounds **3** (8 mg) and **4** (10 mg). Sephadex LH-20 column chromatography of fraction C (1.75 g) eluted with CH₂Cl₂-MeOH (1:1) afforded compounds **5** (125 mg), **6** (50 mg), and **7** (35 mg).

Fraction E (500 mg) was subjected to silica gel column chromatography (*n*-hexane-EtOAc 1:1) to yield compound **8** (45 mg), as well as an inseparable mixture which was repeatedly subjected to silica gel preparative TLC using *n*-hexane-EtOAc 1:1 (triple elution) to afford compound **9** (11 mg). Fraction F (1.5 g) was repeatedly chromatographed on silica gel column chromatography using (*n*-hexane-EtOAc 3:7) as eluent and Sephadex LH-20

column eluted with MeOH to afford compounds **10** (25 mg) and **11** (47 mg). Recrystallization and filtration of fraction G (3.21 g) yielded mainly compound **12** (340 mg).

The *n*-BuOH extract (50 g) was subjected to a silica gel column chromatography using the mixture EtOAc-MeOH with increasing amount of MeOH as eluent to yield four main fractions I-IV. From fraction I (8.3 g), compound **12** (510 mg) was obtained after recrystallization in MeOH. Fraction II (7.1 g) was first submitted to a Sephadex column eluted with MeOH, then to silica gel column chromatography eluted with EtOAc-MeOH-H₂O (95:5:2) to afford compound **13** (50 mg).

Fraction III (10.7 g) was subjected to Sephadex LH-20 column chromatography eluted with MeOH to give a saponins rich mixture which was repeatedly chromatographed using silica gel with EtOAc-MeOH-H₂O (85:15:5) as eluent to afford compounds **14** (345 mg) and **15** (267 mg).

3. RESULTS AND DISCUSSION

Column chromatography of the EtOAc and *n*-butanol soluble fractions of the MeOH extract from the dried roots of *Furcraea bedinghausii* led to the isolation and structure elucidation of a mixture of two new homoisoflavonoids together with the known β -sitosterol (**1**) [7], 7,4'-dihydroxy-homoisoflavane (**2**) [8], dihydrobonducellin (**3**) [9], kaempferol (**5**) [10], 5,7-dihydroxy-3-(4-hydroxybenzyl)-chromone (**6**) [11], 1-linoleylglycerol (**7**) [12], 6'-linoleyl-3-*O*- β -D-glucopyranosyl- β -sitosterol (**8**) [13], *trans*-3,3',5,5'-tetrahydroxy-4'-methoxystilbene (**9**) [5], yuccaol C (**10**) [5,6], yuccaol D (**11**) [6], 3-*O*- β -D-glucopyranosyl- β -sitosterol (**12**) [14], 4-[6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)- β -D-glucopyranosyloxy]-3-methoxybenzoic acid (**13**) [15] and two pairs of steroidal saponins: (25*R*)-2 α -3 β -dihydroxy-5 α -spirostan-12-one 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*-[β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-galactopyranoside (**14a**) [16] and (25*R*)-2 α -3 β -dihydroxy-5 α -spirost-9-en-12-one 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*-[β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-galactopyranoside (**14b**) [16], (25*R*)-3 β -hydroxy-5 α -spirostan-12-one 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*-[β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-galactopyranoside (**15a**) [17] and (25*R*)-3 β -hydroxy-5 α -spirost-9-en-12-one 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*-[β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-galactopyranoside (**15b**) [16] were isolated from the roots of *Furcraea bedinghausii* Koch (Fig. 1).

Compound **4** was obtained as yellow oil in hexane-EtOAc 7:3. It appeared as a mixture of two compounds **4a** and **4b** despite repeated column chromatography on Sephadex and silica gel and the apparent homogeneity on TLC.

The positive mode HRESI-MS showed two pseudomolecular ion peaks at m/z 335.2633 [$M_a + Na$]⁺ and 321.2798 [$M_b + Na$]⁺ corresponding to the molecular formulae C₁₇H₁₂O₆ and C₁₇H₁₄O₅, respectively.

This was confirmed by the negative mode ESI-MS which displayed quasimolecular ion peaks at m/z 311 [$M_a - H$]⁻, 297 [$M_b - H$]⁻, and a characteristic homoisoflavonoid ion fragment at m/z 107 [11].

From this spectrum, it was established that the major compound was **4a** with the quasimolecular ion peak at m/z 311 $[M_a-H]^-$. The IR spectrum indicated the presence of hydroxyl groups ($3600\text{--}3400\text{ cm}^{-1}$), conjugated carbonyls (1658 cm^{-1}), and aromatic rings ($2977, 2947, 1552, 1517\text{ cm}^{-1}$).

The ^1H and ^{13}C -NMR spectra showed a set of duplicated signals corresponding to the common parts of the molecules. However, significant differences were observed, especially for signals corresponding to the B ring. The ^1H -NMR spectrum showed two deshielded proton signals at δ 12.73 and 12.76 ppm corresponding to the chelated protons 5-OH in compounds **4a** and **4b**, respectively (Table 1).

Additionally, it exhibited proton signals at δ 6.31, 6.30, 6.26 and 6.25 ppm, giving correlations in the HSQC spectrum with carbons at δ 93.9, 93.9, 99.3 and 99.5 ppm, characteristic of two homoisoflavonoid units having the C-5 and C-7 dioxygenation patterns [11].

Table 1. ^1H - and ^{13}C -NMR data of compounds **4a** and **4b**, CDCl_3 , multiplicity and J (Hz) in brackets (Fig. 2-4).

Position	4a		4b	
	^{13}C (100 MHz)	^1H (400 MHz)	^{13}C (100 MHz)	^1H (400 MHz)
2	153.3	7.50 (s)	153.3	7.47 (s)
3	123.0	/	123.3	/
4	181.3	/	181.3	/
4a	106.0	/	106.0	/
5	161.7	/	161.6	/
6	99.3	6.26 (d, $J = 2.1$)	99.5	6.25 (d, $J = 2.2$)
7	162.6	/	162.6	/
8	93.9	6.31 (d, $J = 2.1$)	93.9	6.30 (d, $J = 2.2$)
8a	158.2	/	158.2	/
9	30.6	3.67 (s)	30.6	3.70 (s)
1'	131.7	/	131.7	/
2'	108.5	6.75 (brs)	129.9	7.19 (d, $J = 8.6$)
3'	146.3	/	114.1	6.87 (d, $J = 8.6$)
4'	147.8	/	158.4	/
5'	109.3	6.75 (d, $J = 8.5$)	114.1	6.87 (d, $J = 8.6$)
6'	121.9	6.74 (dd, $J = 8.5, 2.0$)	129.9	7.19 (d, $J = 8.6$)
OCH ₂ O	101.0	5.94 (s)		
OMe			55.3	3.79 (s)
5-OH		12.73 (s)		12.76 (s)

Fig. 2. API-ES mass spectrum of compound **4** (**4a** and **4b**).

Fig. 3. ^1H -NMR spectrum of compound 4.

Fig. 4. ^{13}C -NMR spectrum of compound 4.

The ^{13}C -NMR spectrum exhibited some typical signals at δ 181.3 (C-4) and 30.6 ppm (C-9) which were important for the confirmation of the homoisoflavone skeleton of both compounds [11,18]. The major compound **4a** displayed in the ^1H NMR spectrum signals of three protons at δ 6.74 (dd, $J = 8.5, 2.0$, H-6'), 6.75 (brs, H-2') and 6.75 (d, $J = 8.5$, H-5'), characteristic of homoisoflavonoids having a 3',4'-dioxxygenation of the B ring [18]. It also showed a two proton-singlet at δ 5.94 ppm suggesting a methylenedioxy moiety [19]. In the ^{13}C -NMR spectrum, the methylenedioxy group was recognised by the signal at δ 101.0 (OCH₂O). The location of the methylenedioxy group was confirmed by the HMBC correlations observed between its protons at δ 5.94 and carbons at δ 146.3 (C-3') and 147.8 (C-4') ppm (**Fig. 2**). Comparison of the spectroscopic data of **4a** and **4b** revealed that the only difference between them was the substitution patterns of ring B. In compound **4b**, the system constituted by three proton signals was replaced by an AA'BB' spin system characterized by signals at δ 6.87 (d, $J = 8.6$, H-3'/5') and 7.19 (d, $J = 8.6$, H-2'/6'). In the HMBC spectrum, the cross peak correlation observed between the methoxy protons at δ 3.79 ppm (OMe) and the carbon signal at δ 158.4 ppm (C-4') showed that it was linked at (C-4') (**Fig. 2**).

Extensive analysis of ^1H - ^1H COSY, HSQC and HMBC spectra allowed us to assign ^1H and ^{13}C resonances of each component of the mixture (**Table 1**).

Based on the above data, compounds **4a** and **4b** were elucidated as 5,7-dihydroxy-3-(3,4-methylenedioxybenzyl)chromone and 5,7-dihydroxy-3-(4-methoxybenzyl)chromone, new naturally occurring secondary metabolites to which we gave the trivial names furcraeanone A and furcraeanone B, respectively (**Fig. 5**).

Fig. 5. Key HMBC correlations for compounds **4a** and **4b**.

4. CONCLUSION

Steroidal saponins were previously isolated from *Furcraea foetida* [20], *Furcraea selloa* var. *marginata* [3] and *Yucca schidigera* [21]. Furthermore, homoisoflavonoids were previously isolated from plants of the Agavaceae family, especially *Agave sisalana* and *Agave tequilana* [9,22]. 7,4'-dihydroxy-homoisoflavane (**2**), dihydrobonducellin (**3**), and 5,7-dihydroxy-3-(4-hydroxybenzyl)-chromone (**6**) were isolated from *Dracaena cambodiana* [8], *Agave sisalana* [9], and *Dracaena draco* [11], respectively which belong to the families Dracaenaceae and Agavaceae. Agavaceae and Dracaenaceae are placed in Asparagales, an order of the monocotyledoneous plants. The optional synonym of Agavaceae is Asparagaceae and that of Dracaenaceae is also Asparagaceae; showing a clear similarity between the two

families [23,24]. The stilbenoid *trans*-3,3',5,5'-tetrahydroxy-4'-methoxystilbene (**9**) isolated during this work was also obtained from *Yucca schidigera* [5,25] and *Yucca periculosa* [26]. Yuccaols A-E isolated from *Yucca schidigera* [25] possess a very rare spiro-structure. During our investigation on *Furcraea bedinghausii* (*Yucca pringlei*), yuccaols C (**10**) and D (**11**) were obtained. In comparison of the results obtained from *Furcraea bedinghausii* with those obtained from other Agavaceae species, steroidal saponins, homoisoflavonoids and yuccaols could be considered as the chemotaxonomic markers for the family Agavaceae. To the best of our knowledge, this is the first report on the isolation of homoisoflavonoids in a plant of the genera *Furcraea* and *Yucca*.

Acknowledgements

The authors are grateful to the Academy of Sciences for the Developing World (TWAS) for its financial support to the present research through a grant to Dr TEPONNO (RGA N° 09-152 RG/CHE/AF/AC I-UNESCO FR:3240230339).

References

- [1] A. Plock, G. Beyer, K. Hiller, E. Grundemann, E. Krause, M. Nimtz, V. Wray, *Phytochemistry* 57 (2001) 489-496.
- [2] J. C. David, *Hanburyana* 4 (2009) 23-32.
- [3] J. L. Simmons-Boyce, W. F. Tinto, S. McLean, W. F. Reynolds, *Fitoterapia* 75 (2004) 634-638.
- [4] A. Yokosuka, T. Sano, K. Hashimoto, H. Sakagami, Y. Mimaki, *Chem. Pharm. Bull.* 57 (2009) 1161-1166.
- [5] W. Oleszek, M. Sitek, A. Stochmal, S. Piacente, C. Pizza, P. Cheeke, *J. Agric. Food Chem.* 49 (2001) 747-752.
- [6] S. Piacente, P. Montoro, W. Oleszek, C. Pizza, *J. Nat. Prod.* 67 (2004) 882-885.
- [7] R. B. Teponno, A. L. Tapondjou, D. Gatsing, J. D. Djoukeng, E. Abou-Mansour, R. Tabacchi, P. Tane, H. Stoekli-Evans, D. Lontsi, H.-J. Park, *Phytochemistry* 67 (2006) 1957-1963.
- [8] Y. Luo, H. Wang, X. Xu, W. Mei, H. Dai, *Molecules* 15 (2010), 8904-8914.
- [9] P. Y. Chen, Y. C. Kuo, C. H. Chen, Y. H. Kuo, C. K. Lee, *Molecules* 14 (2009) 1789-1795.
- [10] N. Jain, S. Alam, M. Kamil, M. Ilyas, M. Niwa, A. Sakae, *Phytochemistry* 29 (1990) 3988-3991.
- [11] A. G. Gonzalez, F. Léon, L. Sanchez-Pinto, J. I. Padron, J. Bermejo, *J. Nat. Prod.* 63 (2000) 1297-1299.
- [12] B. Thanomsub, T. Watcharachaipong, K. Chotelersak, P. Arunrattiyakorn, T. Nitoda, H. Kanzaki, *J. Appl. Microbiol.* 96 (2004) 588-592.
- [13] S. Ghosal, *Phytochemistry* 24 (1985) 1807-1810.

-
- [14] Y. Mizushina, R. I., Nakanishi, K. Kamiya, T. Satake, N. Shimazaki, O. Koiwai, Y. Uchiyama, Y. Yonezawa, M. Takemura, K. Sakaguchi, H. Yoshida, *J. Steroid. Biochem. Mol. Biol.* 99 (2006) 100-107.
- [15] A. Yokosuka, Y. Mimaki, *Chem. Pharm. Bull.* 55 (2007) 273-279.
- [16] Y. Mimaki, M. Kuroda, A. Kameyama, A. Yokosuka, Y. Sashida, *Phytochemistry* 48 (1998) 1361-1369.
- [17] A. Yokosuka, Y. Mimaki, M. Kuroda, Y. Sashida, *Planta Med.* 66 (2000) 393-395.
- [18] M. Masaoud, H. Ripperger, A. Porzel, G. Adam, *Phytochemistry* 38 (1995) 745-749.
- [19] E. C. N. Nono, P. Mkounga, V. Kuete, K. Marat, P. G. Hultin, A. E. Nkengfack, *J. Nat. Prod.* 73 (2010) 213-216.
- [20] A. Yokosuka, T. Sano, K. Hashimoto, H. Sakagami, Y. Mimaki, *Chem. Pharm. Bull.* 57 (2009) 1161-1166.
- [21] S. Piacente, C. Pizza, W. Oleszek, *Phytochem. Rev.* 4 (2005) 177-190.
- [22] J. A. Morales-Serna, A. Jiménez, R. Estrada-Reyes, C. Marquez, J. Cárdenas, M. Salmón, *Molecules* 15 (2010) 3295-3301.
- [23] The Angiosperm Phylogeny Group, *Bot. J. Linn. Soc.* 161 (2009) 105-121.
- [24] J. L. Reveal, M.W. Chase, *Phytotaxa* 19 (2011) 71-134.
- [26] S. Piacente, G. Bifulco, C. Pizza, A. Stochmal, W. Oleszek, *Tetrahedron Let.* 43 (2002) 9133-9136.
- [26] P. Torres, J. G. Avila, A. Romo de Vivar, A. M. Garcia, J. C. Marin, E. Aranda, C. L. Cespedes, *Phytochemistry* 64 (2003) 463-473.

(Received 05 July 2013; accepted 09 July 2013)