Phytochemistry Letters 10 (2014) 255–259

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Bafouoside C, a new triterpenoid saponin from the roots of *Cussonia bancoensis* Aubrev. & Pellegr.

Beaudelaire K. Ponou [a,b], Raymond N. Nono [a], Rémy B. Teponno [a], Azefack L. Tapondjou [a], Marie-Aleth Lacaille-Dubois [c], Luana Quassinti [d], Massimo Bramucci [d], Luciano Barboni [b,*]

[a] Laboratory of Environmental and Applied Chemistry, Department of Chemistry, Faculty of Science, University of Dschang, Box 183, Dschang, Cameroon
[b] School of Science and Technology, Chemistry Division, University of Camerino, Via S. Agostino 1, I-62032 Camerino, Italy
[c] Laboratoire de Pharmacognosie, UFR des Sciences de Santé (Pharmacie), EA 4267 (FDE/UFC), Université de Bourgogne, 7, Boulevard Jeanne d'Arc, BP 87900, 21079 Dijon Cedex, France
[d] School of Pharmacy, Physiology Division, University of Camerino, Via Gentile III da Varano, I-62032 Camerino, Italy

ARTICLE INFO

Article history:
Received 11 July 2014
Received in revised form 3 October 2014
Accepted 6 October 2014
Available online 22 October 2014

Keywords:
Cussonia bancoensis
Araliaceae
Saponins
Oleanane triterpenoid

ABSTRACT

A new triterpenoid saponin named bafouoside C 3-*O*-β-D-glucopyranosyl-(1 → 4)-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyloleanolic acid 28-*O*-β-D-glucopyranosyl ester; (1), together with five known compounds 3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyloleanolic acid (2), 23-hydroxyursolic acid (3), 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl-23-hydroxyursolic acid (4), 3-*O*-β-D-glucopyranosyl-23-hydroxyursolic acid (5), and 3-*O*-α-L-arabinopyranosyl-23-hydroxyursolic acid (6), were isolated from the roots of *Cussonia bancoensis* Aubrev. & Pellegr. Their structures were established on the basis of 1D- and 2D NMR data, mass spectrometry and chemical methods. The NMR data of the known compounds, as far as we know, are herein reported for the first time in CD_3OD. Compound 3 exhibited a weak cytotoxic activity against MDA-MB 231 human breast adenocarcinoma, A375 human malignant melanoma, and HCT116 human colon carcinoma cell lines.

1. Introduction

The genus *Cussonia* (Araliaceae) comprises 21 species found in grasslands, woodlands and forests of sub-Saharan Africa, the Arabian Peninsula (Yemen) and the Comoro Islands. *Cussonia* species are traditionally used in the treatment of various diseases such as eyes injuries, paralysis, epilepsy, gastro-intestinal problems, women's infertility, diarrhea, stomach ulcers and syphilis (Watt and Breyer-Brandwijk, 1962; Hardi, 1964; Bouquet and Debray, 1974; Burkhill, 1985; Harinantenaina et al., 2002). Scientific results have previously supported the traditional use of *Cussonia* species for the treatment of sexually transmitted diseases and *Plasmodium* infections (De Villiers et al., 2010). They are also known as a rich source of pentacyclic triterpenoids and their glycoside derivatives, mainly with oleanane and ursane type skeletons (Dubois et al., 1986; Tapondjou et al., 2003; Grishkovets et al., 2005; Kougan et al., 2009). Ursolic and oleanolic acid are recognized to have significant anti-tumor activity (Liu, 1995). They have been shown to act at various stages of tumor development to inhibit tumor initiation and promotion, as well as to induce tumor cell differentiation and apoptosis. Oleanolic acid derivatives are also effective for acute myeloid leukemia by inducing apoptosis of tumor cells (Konopleva et al., 2004). These triterpenoids and their derivatives are also effective in inhibiting angiogenesis, invasion of tumor cells and metastasis (Oguro et al., 1998). *Cussonia bancoensis* Aubrev. & Pellegr. (The Plant List, 2013) is used in the Nigeria folk medicine for the treatment of dizziness, women's infertility, wound healing and sexually transmitted diseases (Adjanohoun et al., 1991). Previous phytochemical studies of the methanol stem bark extract of this plant led to the isolation of triterpenoids and their glycoside derivatives (Tapondjou et al., 2003).

As part of our continuous effort to isolate bioactive saponins from Cameroonian medicinal plants (Ponou et al., 2008, 2010; Nzowa et al., 2010; Tapondjou et al., 2011; Fouedjou et al., 2014), we have examined the methanol extract from the roots bark of *C. bancoensis*. In the present article we describe the isolation and the structure elucidation a new oleanane-type triterpenoid saponin, 3-*O*-β-D-glucopyranosyl-(1 → 4)-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosyloleanolic acid 28-*O*-β-D-glucopyranosyl ester (**1**), together with the known

* Corresponding author. Tel.: +39 0737402240; fax: +39 0737402297.
E-mail address: luciano.barboni@unicam.it (L. Barboni).

http://dx.doi.org/10.1016/j.phytol.2014.10.007
1874-3900/

3-*O*-*β*-D-galactopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyloleanolic acid (**2**) (Grishkovets et al., 1997), 23-hydroxyursolic acid (**3**) (Tapondjou et al., 2003), 28-*O*-*α*-L-rhamnopyranosyl-(1 → 4)-*O*-*β*-D-glucopyranosyl-(1 → 6)-*O*-*β*-D-glucopyranosyl-23-hydroxyursolic acid (**4**) (Dovgii et al., 2005), 3-*O*-*β*-D-glucopyranosyl-23-hydroxyursolic acid (**5**) (Tapondjou et al., 2003), and 3-*O*-*α*-L-arabinopyranosyl-23-hydroxyursolic acid (**6**) (Tapondjou et al., 2003). The isolated compounds were tested against a selection of human tumor cell lines as MDA-MB 231 human breast adenocarcinoma cell line, A375 human malignant melanoma cell line, and HCT116 human colon carcinoma cell line.

2. Results and discussion

2.1. Structure elucidation

The crude methanol extract of the roots bark of *C. bancoensis* was repeatedly subjected to column chromatography to afford six compounds (**1**–**6**) (Fig. 1).

Compound **1** was isolated as a white amorphous powder from the *n*-BuOH fraction. It gave positive results to the Liebermann–Burchard and Molisch tests, revealing it as a triterpenoid saponin. Its molecular formula $C_{54}H_{86}O_{24}$ was established by the negative ion mode HRESIMS which showed the quasimolecular ion peak at *m*/*z* 1153.5203 $[M+Cl]^-$ (calcd for $C_{54}H_{86}O_{24}Cl$: 1153.5198). This was confirmed by the ESIMS, which exhibited a quasi-molecular ion peak at *m*/*z* 1117.4 $[M-H]^-$. Another important ion peak observed at *m*/*z* 617.4 $[M-2\times162-176]^-$ was indicative for the loss of three hexose moieties including hexosyluronic acid. On the basis of ^1H and ^{13}C NMR spectral data (Table 1), compound **1** was identified as an olean-12-ene type pentacyclic triterpene saponin, and this was confirmed by comparison of its NMR data with those of known olean-12-ene derivatives (Mahato and Kundu, 1994).

The ^1H NMR spectrum showed seven tertiary methyl signals (*δ* 0.80, 0.84, 0.90, 0.93, 0.95, 1.05 and 1.15) and one olefinic proton signal at *δ* 5.26 (br *s*, H-12), one oxygen-bearing methine proton at *δ* 3.17 (*dd*, *J* = 3.9, 11.7, H-3). The ^{13}C NMR spectrum showed one signal of a carbonyl ester group at *δ* 176.6 (C-28), as well as two olefinic carbon atom signals at *δ* 122.3 (C-12) and 143.4 (C-13). The aglycone was identified as oleanolic acid and all the ^1H and ^{13}C NMR spectral data were in good agreement with literature values (Sarikaya and Kirmizigül, 2010). Also, signals of four anomeric proton doublets at *δ* 4.46 (*d*, 7.5, H-1′), 4.62 (*d*, 7.8, H-1‴), 4.73 (*d*, 7.5, H-1″) and 5.38 (*d*, 7.3, H-1⁗) were observed, giving HSQC correlations with four anomeric carbons at *δ* 103.7, 104.7, 103.0 and 93.7, respectively. The coupling constant observed for the anomeric protons were indicative of sugars with *β*-configurations (Huan et al., 1998). Extensive 2D NMR analysis and evaluation of the spin-spin coupling constants and chemical shifts of the sugar part allowed the identification of one *β*-glucuronopyranosyl (Glc A), two *β*-glucopyranosyl (Glc) and one *β*-galactopyranosyl (Gal) units. Extensive survey of triterpenoid saponins showed that, when the sugar part contains a *β*-glucuronic acid, this one is preferably linked to C-3 of the aglycone (Huan et al., 1998; Gaidi et al., 2001; Mshvildadze et al., 2001; Luo et al., 2008; Tuyet et al., 2009). In the particular case of triterpenoid saponins with oleanolic acid as aglycone, this carbon (C-3 of the aglycone) resonates at about 89.6 ppm (Kawai et al., 1989; Huan et al., 1998; Tuyet et al., 2009). The downfield shift observed for C-3 (*δ* 89.6) and the upfield shift at *δ* 176.6 for C-28 (Table 1) reflected the bidesmosidic nature of compound **1**. Furthermore, the HMBC correlations observed between the anomeric proton atoms at *δ* 4.46 (H-1′) and 5.38 (H-1‴) and carbon atoms at *δ* 89.6 (C-3) and 176.6 (C-28), respectively evidenced the linkage positions of the sugar chains. The downfield shift observed for C-2′ (*δ* 82.1) and C-4′ (*δ* 82.0) were indicative for the 2,4-glycosylation of the glucuronopyranosyl moiety (Tuyet et al., 2009), and this was further supported by HMBC cross peak correlations observed between anomeric proton atoms at *δ* 4.62 (H-1‴), 4.73 (H-1″) and the carbons at *δ* 82.0 (C-4′) and 82.1 (C-2′), respectively. The remaining glucopyranosyl unit was then attached to C-28 of the aglycone. Selected COSY and HMBC correlations for compound **1** are reported in Fig. 2.

The identification of the sugars was also confirmed through partial acid hydrolysis followed by co-TLC in comparison with standard sugars, and their absolute configuration was determined to

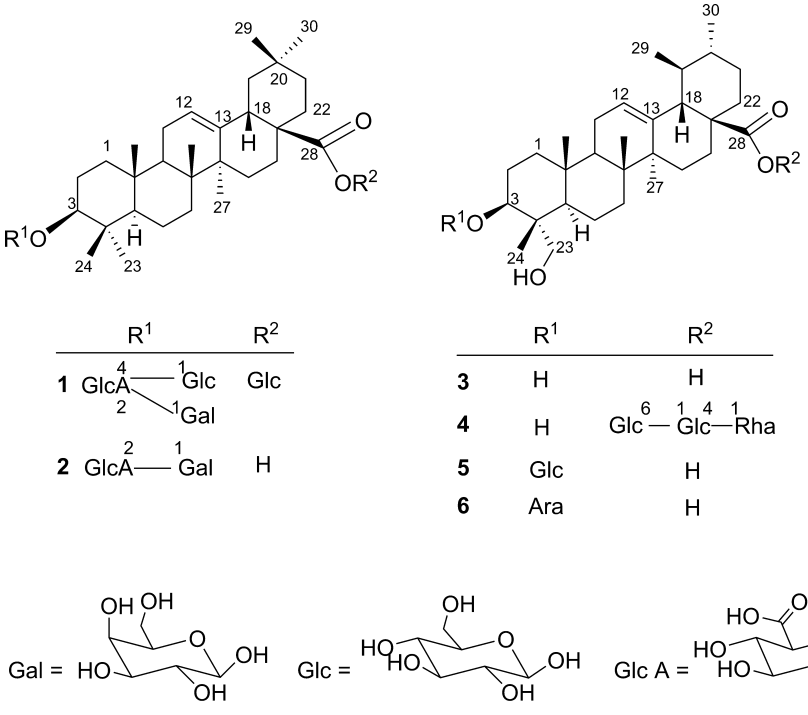

Fig. 1. Structures of compounds **1**–**6**.