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Acylated pregnane glycosides from *Caralluma sinaica*

Shaza M. Al-Massarani [a], Samuel Bertrand [b], Andreas Nievergelt [b], Azza M. El-Shafae [a], Tawfeq A. Al-Howiriny [a], Nawal M. Al-Musayeib [a], Muriel Cuendet [b], Jean-Luc Wolfender [b,*]

[a] King Saud University, College of Pharmacy, Dept. of Pharmacognosy, P.O. Box 2457, Riyadh 11451, Saudi Arabia
[b] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 30 quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland

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ABSTRACT

Caralluma sinaica is sold on local markets of Saudi Arabia for various health benefits however no phytochemical study has specifically been performed on this species. NMR and UHPLC-ESI-TOF-MS profilings of the ethanolic extract of the whole plant reveal a very complex phytochemical composition dominated by pregnanes. Detailed information on its constituents was obtained after isolation. Six pregnane glycosides were obtained and characterized based on the extensive spectroscopic analysis (including IR, ^{1}H NMR, ^{13}C NMR and MS data), in addition to ten known compounds (seven pregnanes and three flavonoids). The compounds were identified as 12β-*O*-benzoyl-20-*O*-acetyl boucerin-3-*O*-6-deoxy-3-*O*-methyl-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, 12β-*O*-tigloyl-20-*O*-acetyl boucerin-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranoside, 12β-*O*-benzoyl-20-*O*-acetyl boucerin-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-digitalopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, 12β-*O*-benzoyl-20-*O*-acetyl boucerin-3-*O*-β-D-glucopyranosyl-(1→4)-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, 12β-*O*-benzoyl-20-*O*-tigloyl boucerin-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranoside, 12β-20-*O*-dibenzoyl boucerin-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside. Finally, the isolated compounds were evaluated for their quinone reductase induction.

1. Introduction

The genus *Caralluma* belongs to the Asclepiadaceae family, which is also known as the milkweed family because many of its members contain a milky latex (Bensuzan, 2009). Due to recent DNA analysis and morphological studies, Asclepiadaceae have been classified as a sub-group of the family Apocynaceae (Endress and Bruyns, 2000; Meve and Heneidak, 2005). Nevertheless, Asclepiadaceae is still regarded as an independent family. Plants of the genus *Caralluma* are perennial, small and usually leafless (Heyood, 1978; Saxena and Sarbhai, 1975). Some of these plants are edible and succulent (Marwah et al., 2007; Reddy et al., 2011). More than 200 species of the genus *Caralluma* grow throughout Africa and Asia (Surveswaran, 2007). The majority of these species are indigenous to the Indian sub-continent and Arabian peninsula (Gilbert, 1990).

Various medicinal uses of *Caralluma* spp. have been reported in Arabic and Indian traditional medicine such as treatment of cancer, diabetes, tuberculosis, snake and scorpion bites, skin rash, scabies, fever and inflammation (Abdel-Sattar et al., 2007; De Leo et al., 2005; Oyama et al., 2007; Ramesh et al., 1999; Western, 1986). Because of its claimed appetite suppressant activity, *Caralluma fimbriata* encounters an important interest from the public at large and is the widely commercially available *Caralluma* species at present (Kuriyan et al., 2007; MacLean and Luo, 2004). *Caralluma sinaica* (Decne.), which is the species considered for this study, is only sold in local markets and is reputed to have aphrodisiac, anti-diabetic and anti-cancer activities (Habibuddin et al., 2008).

Previous phytochemical and biological investigations of the genus *Caralluma* led to the isolation of several pregnane, flavone and megastigmane glycosides, as well as triterpenes (Bader et al., 2003; Braca et al., 2002; Muller and Albers, 2002). Notably, numerous polyhydroxy pregnane ester glycosides with significant antitumor activity were isolated from several members of the family Asclepiadaceae (Braca et al., 2002; Chen et al., 2010; Halaweish et al., 2004; Li et al., 2008; Plaza et al., 2005).

While *C. sinaica* is a commonly used plant in Saudi Arabia (Habibuddin et al., 2008), to our knowledge, it has not yet been investigated in details from a phytochemical viewpoint. The scope of our study was to explore the chemical composition of this plant in relation to other *Caralluma* species and plants from the Asclepiadaceae, to document the bioactivity of some of their constituents. In order

* Corresponding author. Address: Phytochimie et Produits Naturels Bioactifs, Ecole de Pharmacie Genève-Lausanne, Section des Sciences Pharmaceutiques, Université de Genève, Quai Ansermet 30, 1211 Genève 4 Switzerland. Tel.: +41 22 379 33 85; fax: +41 22 379 33 99.
E-mail address: jean-luc.wolfender@unige.ch (J.-L. Wolfender).

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to obtain a rather comprehensive view of the *C. sinaica* metabolome and check its potential for new compounds, the extract was profiled by NMR and high resolution (HR) ultra high pressure liquid chromatography–mass spectrometry (UHPLC–MS). The present study focusses on the isolation and complete characterization of polyhydroxy pregnane ester glycosides along with some flavonoids by using 1D and 2D NMR spectroscopy and HR-MS. The quinone reductase induction of the isolated compounds was also assessed.

2. Results and discussion

2.1. NMR and UHPLC-ESI-TOF-MS profiling of C. sinaica extract

In order to obtain most of the constituents of *C. sinaica* of medium polarity, the plant was extracted with ethanol according to an established protocol (Khalil, 1995). Both NMR (Verpoorte et al., 2007; Wolfender et al., 2010) and UHPLC-MS (Eugster et al., 2011) profilings were performed on this crude extract and compared with references to all previously reported compounds from the *Caralluma* genus.

This ethanolic extract was directly dissolved in deuterated methanol and profiled by NMR. The ^{1}H- and gHSQC-NMR spectra (Fig. 1A and B) showed various glycosylated compounds through the ^{1}H–^{13}C–OH signal in the (3–4 ppm and 60–90 ppm in ^{1}H- and ^{13}C-NMR, respectively) region and the corresponding typical anomeric protons (4–5 ppm and 90–110 ppm).

The presence of various signals in the aliphatic proton region (1–3 ppm and 10–50 ppm) confirms the presence of steroidal compounds which can be putatively assigned to pregnanes by studying previous reported data on *Caralluma* species (Abdel-Sattar et al., 2007; De Leo et al., 2005; Halaweish et al., 2004; Kunert et al., 2009; Qiu et al., 1999; Reddy et al., 2011; Waheed et al., 2011). The aromatic proton signals at 8.05 ppm (2H, *dd*, *J* = 1.25, 8.45), 7.57 ppm (1H, *t*, 7.45) and 7.46 ppm (2H, *t*, 7.73) can be attributed to the typical pattern of mono-substituted phenyl groups. This is also in good agreement with previous reports on acylated pregnanes. The comparison of the gHSQC-NMR spectrum from the crude extract (Fig. 1B) with the one obtained with a known pregnane glycoside (russelioside G, Fig. 1C) confirms the presence of pregnanes in the ethanolic extract (Tanaka et al., 1990). Since almost no additional signal could be detected, this also indicated that this crude extract is largely dominated by this type of compounds.

The presence of steroid glycosides was also confirmed by the positive reaction to Libermann–Buchard and Keller–Kiliani tests performed on the crude extract (Li et al., 2006).

In order to confirm this hypothesis, the crude extract was hydrolysed after enrichment, and the NMR spectrum revealed the presence of two aglycones, namely boucerin and caralumagenin by comparison with literature data (Abdel-Sattar et al., 2008; Halim and Khalil, 1996; Lee-Juian et al., 1994).

To obtain a more detailed view and get an idea of the diversity of all pregnane glycosides present, the extract was profiled by high resolution UHPLC combined with time of flight mass spectrometry (TOF-MS) (Grata et al., 2009). The chromatogram and corresponding ion map generated in the negative ion mode (NI) revealed an extremely complex composition (Fig. 2). The automatic peak detection at a threshold level of 5% indicated that 40 features of more than 500 Da could be detected and this number was over a hundred when the intensity threshold was lowered to 1%. The molecular formula of all these compounds was determined directly from the TOF-MS data generated on the extract. The combination of high mass accuracy (5 ppm) and heuristic filters (Kind and Fiehn, 2007) provided putative formulae that matched well with glycosylated pregnanes. Based on this preliminary information

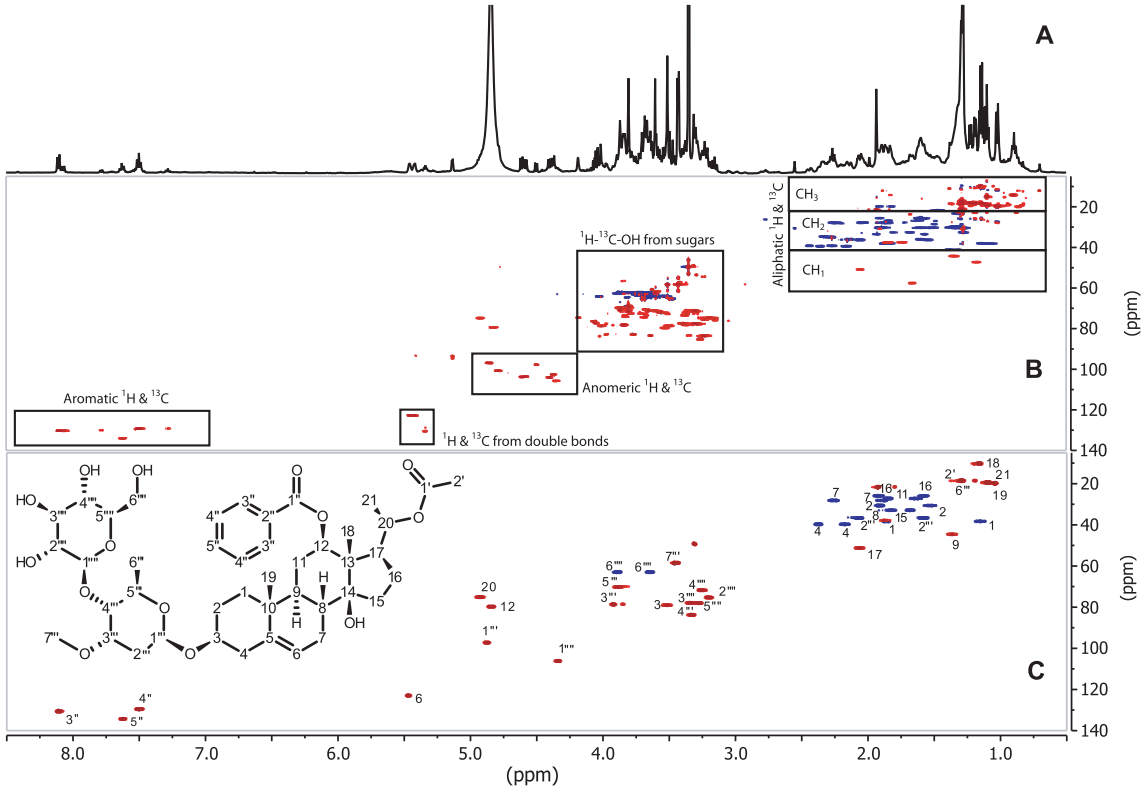

Fig. 1. ^{1}H- and gHSQC-NMR spectra (CD_3OD) of *C. sinaica* ethanolic extracts (A and B) and gHSQC of russelioside G (C). The gHSQC spectrum of the crude extract is highly similar to that of the boucerin derivative showing the high content in pregnane of the *C. sinaica* extract.