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Acylated pregnane glycosides from *Caralluma quadrangula*

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ABSTRACT

In a previous study, the methanolic extract as well as the chloroform fraction of the aerial parts of *Caralluma quadrangula* (Forssk.) N.E.Br. indigenous to Saudi Arabia showed significant *in vitro* cytotoxic activity against breast cancer (MCF7) cell line. In a biologically-guided fractionation approach, four acylated pregnane glycosides were isolated from the chloroform fraction of *C. quadrangula*. The structures of the isolated compounds were elucidated by the analysis of their MS and NMR data. The compounds were identified as 12,20-di-*O*-benzoylboucerin 3-*O*-β-D-digitoxopyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-cymaropyranoside (**1**), 12,20-di-*O*-benzoylboucerin 3-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-cymaropyranoside (**2**), 12,20-di-*O*-benzoylboucerin 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-cymaropyranoside (**3**) and 12,20-di-*O*-benzoyl-3β,5α,12β,14β,20-pentahydroxy-(20*R*)-pregn-6-ene 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-canaropyranosyl-(1 → 4)-β-D-cymaropyranoside (**4**). The isolated compounds were tested for their cytotoxic activity against breast cancer (MCF7) cell line.

1. Introduction

In a continuation of our interest in the chemical and biological investigation of the genus *Caralluma* (Abdel-Sattar et al., 2001, 2007, 2008; Al-Yahya et al., 2000), the aerial parts of *Caralluma quadrangula* (Forssk.) N.E.Br. indigenous to Saudi Arabia were investigated (Collenette, 1999). The plant has been used by Bedouins of Saudi communities in cases of thirst and hunger and for the treatment of diabetes, vitiligo, melasma and freckles (Gushash, 2006). In the present study, we report on the isolation and *in vitro* cytotoxic activity of four acylated pregnane glycosides from the chloroform fraction of *C. quadrangula*.

2. Results and discussion

In the course of screening program for biologically active natural products, the methanolic extracts of forty species of plants traditionally used in Saudi traditional medicine for the treatment of a variety of diseases were previously tested *in vitro* for their potential cytotoxicity on different human cancer cell lines (Almehdar et al., 2012). In a biologically-guided fractionation approach, the methanolic extract of *C. quadrangula* as well as, chloroform, *n*-butanol and remaining aqueous fractions were tested for their cytotoxic activity against three human cancer cell lines, namely, breast cancer (MCF7), hepatocellular carcinoma (HEPG2), and cervix cancer (HELA) cells. In addition, human normal melanocyte (HFB4) was used as normal non-malignant cells (Almehdar et al., 2012). In the present study, four acylated pregnane glycosides were isolated from the active chloroform fraction (IC_{50} 5.59 μg/ml on MCF7 human cancer cell line) through a bio-guided fractionation approach.

The chloroform soluble fraction was subjected to chromatographic separation on normal, reversed phase (RP-18) Si gel columns and Prep HPLC to provide four compounds (**1**–**4**). The isolated compounds (Fig. 1) were tested for their cytotoxicity on MCF7 human cancer cell line.

2.1. Identification of pregnanes from C. quadrangula

Compounds **1**–**4** showed positive Libermann–Buchard and Keller–Kiliani reactions indicating the presence of steroidal skeleton with a 2-deoxy sugar moiety (Li et al., 2006). A detailed spectroscopic analysis of compounds **1**–**4** (Table 1) and by comparison to the data of the previously isolated pregnane glycosides from genus *Caralluma*, allowed the identification of the aglycone of compounds **1**–**3** as the C/D-*cis*-polyhydroxy pregnane; boucerin

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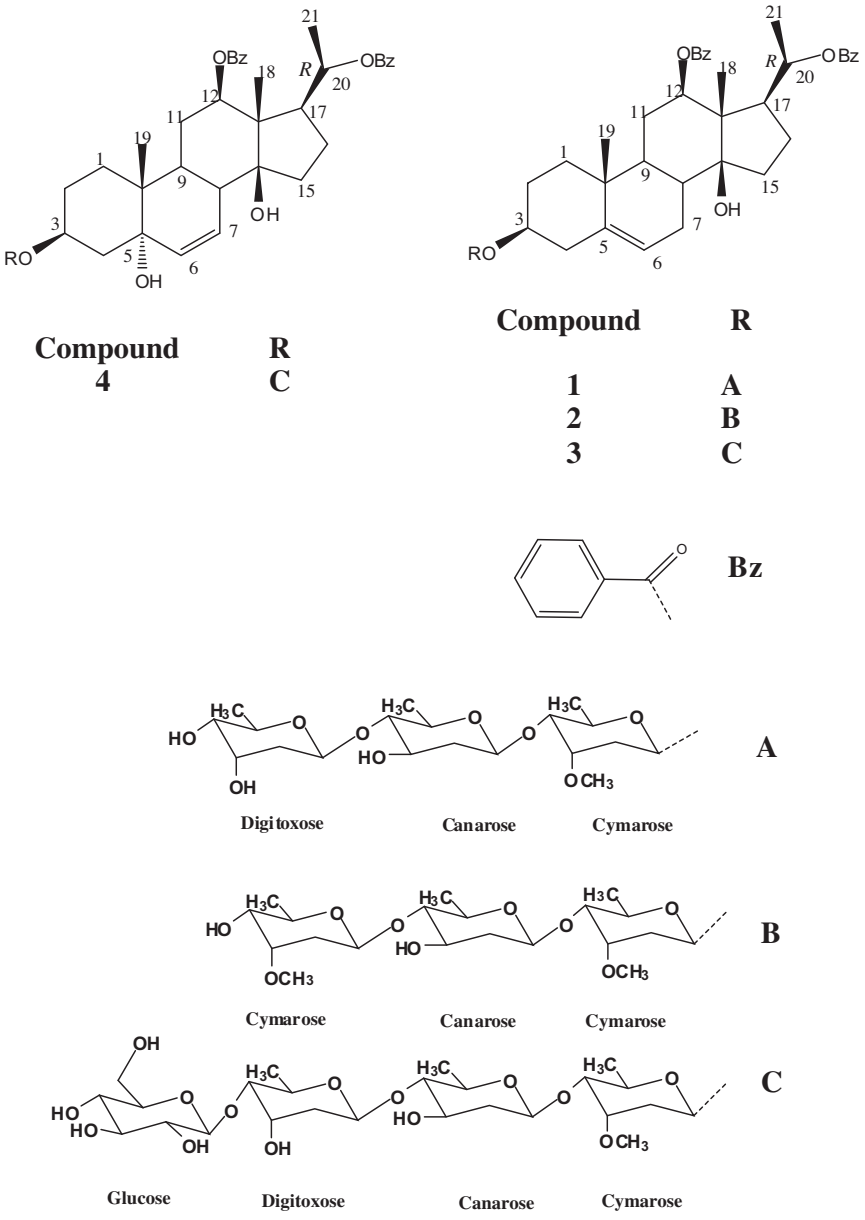

Fig. 1. Structures of compounds **1**–**4**.

(3β,12β,14β,20-tetrahydroxy-(20*R*)-pregn-5-ene (Abdel-Sattar et al., 2007; Al-Massarani et al., 2012; Braca et al., 2002; Kunert et al., 2009). Compounds **1**–**4** showed the presence of two benzoyl groups at C-12 and C-20 positions and a straight sugar chain consisting of three to four sugar units connected to C-3 of the aglycone. The acylation at C-12 and C-20 was confirmed from the downfield shift of their corresponding protons and carbons and from the results of HMBC experiments (Fig. 2) which showed clear long-range correlation between the signal of carbonyl carbons of benzoyl groups and H-12 and H-20 of the aglycone moiety (Abdel-Sattar et al., 2007; Braca et al., 2002; Kunert et al., 2009). The relative stereochemistry at the chiral centers of the aglycone moiety was deduced from NOESY (Fig. 2) experiments and by comparison of the chemical shifts of the carbons and protons coupling constants with those reported for related pregnanes (Ahmad et al., 1988; Basha and Ahmad, 2007; Panda et al., 2003).

The identification of the monosaccharides in the hydrolysates of $CHCl_3$ extract was confirmed by co-TLC comparison with authentic sugars. The absolute configuration of the deoxy sugars was determined to be D-forms by comparison with authentic samples on TLC, ^{13}C NMR data and optical rotation with the reported data (Abe and Yamauchi, 2000).

A survey of closely related glycosides from Asclepiadaceae family (Vleggaar et al., 1993) revealed that all the β-linked 2,6-dideoxy sugars have the D configuration, whereas the α-linked sugars are mostly L-sugar. An analysis of the ^{13}C chemical shift values for C-2 of 2-deoxy sugar (cymaropyranose and digitoxopyranose) of a large number of steroid glycosides revealed that C-2 of β-D-sugars resonates at δ_C 35–38 and that of α-D-sugaras at δ_C 35–36 whereas the corresponding carbon atom of α-L-sugar appears in the δ_C 30.0–32.0 region. The chemical shift values for C-2 of cymaropyranose and digitoxopyranose moieties (Table 2) confirmed their D-configuration (Vleggaar et al., 1993) together with their optical rotation data. Due to lake of authentic canarose, its data were compared to literature which was typical D-configuration.

All sugar linkages in the glycosides were assigned to be in the β-form based on their large coupling constants of the anomeric protons as shown in Table 2, J = 9.6 and 2.0 Hz for cymaropyranose;