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Triterpenoid saponins from the roots of *Gypsophila trichotoma* Wender.

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ABSTRACT

Eleven triterpenoid saponins were isolated from the roots of *Gypsophila trichotoma* Wender. (*G. trichotoma* Wender. var. *trichotoma*) (Caryophyllaceae), together with one known compound. The structures were established on the basis of extensive NMR analysis (^{1}H, ^{13}C NMR, COSY, TOCSY, ROESY, HSQC, and HMBC), completed by analysis of HR-ESI-MS and ESI-MS^{n}. The saponins have the commonly found gypsogenin as the aglycone substituted at C-3 with trisaccharide and at C-28 with oligosaccharide through a fucose residue, as saponins isolated from *Gypsophila perfoliata* L. originated from China. The oligosaccharide attached to C-28 is substituted with acetyl and (or) sulfate groups.

The cytotoxicity of the saponin extract from *G. trichotoma* was evaluated against a rat alveolar macrophage-like cell line NR8383 and human leukemia cell lines U937 and BV-173. The synergistic effect of the aminoacyl saponins, previously isolated from *G. trichotoma*, was tested for its ability to enhance the cytotoxicity of the targeted toxin in HER14 cells.

1. Introduction

Triterpenoid saponins from the genus *Gypsophila*, belonging to the Caryophyllaceae family, are exploited commercially for a variety of purposes including as medicines, detergents, adjuvants, cosmetics (Tschesche and Wulff, 1973; Hostettmann and Marston, 1995). A large number of the *Gypsophila* mono- and bidesmosides have been isolated and characterized (Frechet et al., 1991; Henry et al., 1991; Kim et al., 1992; Liu et al., 1995; Elgamal et al., 1995; Acebes et al., 1998; Luo and Kong, 2006; Luo et al., 2006, 2008; Bai et al., 2007; Zheng et al., 2007; Elbandy et al., 2007a,b; Cevrimli et al., 2007; De Abreu et al., 2008; Nie et al., 2009; Weng et al., 2009, 2010; Yao et al., 2010; Chen et al., 2010, 2011). The most common basic structures are gypsogenin, gypsogenic acid and quillaic acid (Bottger and Melzig, 2010), as well as vaccaric acid (Luo et al., 2006), segetalic acid (Luo et al., 2008) and the new sapogenins neogypsogenin A and B (Luo and Kong, 2006). Saponins are considered as the major bioactive components of the drugs, mainly used for their anti-inflammatory, spermicidal (El Bary and Nour, 1979; Primorac et al., 1985), hypocholesterolaemic (Velieva et al., 1988), and antiviral (Dourmashkin et al., 1962; Frechet et al., 1990) activities. Some saponins from *Gypsophila* species have been reported to exert immunomodulant activities (Luo et al., 2006). Also, saponins with an aldehyde function at C-4 from *Gypsophila oldhamiana* Miq. exhibited cytotoxic activity against different human cancer cell lines (Bai et al., 2007). *Gypsophila* saponins are of interest in terms of their applications as immunological adjuvants in vaccines (Marciani, 1999). It was shown that saponinum album (Merck) enhanced 100 000-folds the cytotoxicity of the type I ribosome – inactivating protein saporin from *Saponaria officinalis* L. (Hebestreit et al., 2006). Individually isolated *Gypsophila paniculata* L. saponins showed also toxicity enhancing properties on saporin without causing toxicity by themselves up to 20 μg/ml (Weng et al., 2009, 2010). The principle of the synergistic cytotoxicity was adopted for saporin-based targeted toxins which are used in tumor therapy (Bachran et al., 2010; Bottger and Melzig, 2010). Especially saponins of *G. oldhamiana* Miq. and *G. paniculata* L. showed inhibitory activity against α-glucosidase (Luo et al., 2008). This effect might offer the use of saponins found in some species as an antidiabetic remedy. Besides, saponins from *G. oldhamiana* have been reported to exert inhibitory activity of pancreatic lipase (Zheng et al., 2007).

The medicinal and commercial importance attached to *Gypsophila* prompted us to investigate the saponins in *Gypsophila trichotoma* Wender. (synonym of *G. trichotoma* Wender. var. *trichotoma*) (Valev, 1966). This perennial herb is native to Black Sea region in Bulgaria

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(Melchior, 1964). Previous phytochemical studies on this species have reported the occurrence of saponins, sterols and flavonoids (Luchanskaya et al., 1971a,b,c; Luchanskaya et al., 1972; Krasteva et al., 2009). In order to found reliable source of hemolytic saponins and vaccine adjuvants, we previously carried out an investigation on *G. trichotoma* Wender., which resulted in the isolation of two new aminoacyl triterpene saponins (Gevrenova et al., 2006). 3-*O*-β-sulfated glycosides were already reported from the roots of *Gypsophila bermejoi* G. Lopez, *Gypsophila repens* L. and *G. trichotoma* Wender (Acebes et al., 1998; Elbandy et al., 2007a; Krasteva et al., 2009). Recently, new triterpenoid saponins with an acetyl group or a cinnamoyl group were isolated from the roots of *Gypsophila altissima* L. and *Gypsophila perfoliata* L. (Chen et al., 2010; Chen et al., 2011). The taxonomy of *G. trichotoma* is ambiguous and this species is synonym of *G. perfoliata* L. in Flora Europaea (Barkoudah and Chater, 1996) and Flora of China (Lu and Turland, 2001). According to Flora Bulgaricae, only *G. trichotoma* Wender. var. *pubescens* Fenzl. is synonym of *G. perfoliata* L. (Valev, 1966). The present study describes the isolation and structural elucidation of 11 new *Gypsophila* saponin (**1**–**11**) from the roots of *G. trichotoma* Wender. var. *trichotoma*, as well as the cytotoxicity of the saponin extract against a rat alveolar macrophage – like cell line NR8383 and human leukemic cell lines U937 and BV-173. The synergistic effect of the aminoacyl saponins, previously isolated from *G. trichotoma*, was also tested for its ability to enhance the cytotoxicity of the targeted toxin in HER14 cell cultures.

2. Results and discussion

2.1. Isolation and identification of new compounds *1*–*11*

The hydroalcoholic extracts of *G. trichotoma* roots were purified by multiple chromatographic steps over silica gel, Reversed phase C_8, Reversed phase C_{18} and finally purified by semi-preparative HPLC over C_{18}, yielding 11 new saponins (**1**–**11**, Fig. 1) with a known saponin (**12**), the 3-*O*-β-D-xylopyranosyl-(1 → 3)-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-xylopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-4-*O*-acetyl-fucopyranoside already reported in the seeds of *Agrostemma githago* L. var. *githago* (Siepmann et al., 1998). Among them, saponins **9** and **11** were obtained in a poor quantity to obtained ^{13}C NMR data.

Acid hydrolysis of crude saponin mixture afforded an aglycone identified, after purification, as gypsogenin from ^1H and ^{13}C NMR spectra referring to the reported data (Mahato and Kundu, 1994) and a prosaponin identified as 3-*O*-β-D-6-*O*-methylglucuronopyranosyl-gypsogenin (**13**), previously isolated after hydrolysis of *Momordica* saponin (Iwamoto et al., 1985). This prosaponin may be considered as a hydrolysis artefact of prosaponin gypsogenin 3-*O*-β-D-glucuronopyranoside (Henry et al. 1989). Seven sugar moieties were identified in the aqueous layer by co-TLC with authentic samples to glucuronic acid (GlcA), glucose (Glc), galactose (Gal), fucose (Fuc), arabinose (Ara), xylose (Xyl) and rhamnose (Rha). The common D configuration for GlcA, Glc, Gal, Xyl and Fuc and the L configuration for Rha and Ara were assumed after measurement of their optical rotation after purification of each sugar by preparative TLC, and corresponded to the D and L configurations previously characterized in the structural elucidation of trichoside D (Luchanskaya et al., 1972).

Compound **1** was obtained as a white amorphous powder. The HR-ESI-MS (negative ion mode) exhibited pseudomolecular ion peaks at *m*/*z* 1647.6368 $[M-H]^-$, (calcd for $C_{72}H_{111}O_{40}S$, 1647.6372) indicating a molecular mass of 1648 Da, compatible with the molecular formula $C_{72}H_{112}O_{40}S$. This pseudomolecular ion was observed in ESI-MS^- at *m*/*z* 1647 with other fragments ions peaks at *m*/*z* 1568 $[M-HSO_3]^-$, 1177 $[M-H-470]^-$ and 939 $[M-H-708]^-$, due to the loss of one sulphated group and two glycosidic chains, respectively. The chain with 470 *uma* was composed with hexose, pentose and hexosuronic acid. Another fragment ion peaks was observed at *m*/*z* 707 corresponding to the ion fragment of the ester chain (707 *uma*). These ions suggest that the ester glycosidic chain was constituted by four sugars, two desoxyhexoses, one hexose and one pentose substituted by one sulphated group and one acetyl group. The proton and carbon resonances of the aglycon moiety of saponin **1** were unambiguously assigned to the gypsogenin from the analysis of COSY, HSQC *J*-modulate and HMBC NMR spectra (Table 1), and by comparison with literature data (Mahato and Kundu, 1994). The chemical shift of the carbonyl C-28 (δ_C 178.2) and the deshielded carbon C-3 (δ_C 86.7) of gypsogenin indicated a bidesmosidic saponin. Seven anomeric proton signals were observed at δ_H 5.42 (1H, *d*, *J* = 8.1 Hz), 5.18 (1H, *d*, *J* = 1.1 Hz), 4.84 (1H, *d*, *J* = 7.4 Hz), 4.70 (1H, *d*, *J* = 8.0 Hz), 4.68 (1H, *d*, *J* = 8.0 Hz), 4.59 (1H, *d*, *J* = 7.4 Hz), and 4.49 (1H, *d*, *J* = 7.7 Hz), displaying correlations with anomeric carbon signals at δ_C 95.3, 102.1, 103.7, 105.2, 105.0, 104.8, and 104.6, respectively (Table 2). Analysis of COSY, TOCSY, and HSQC *J*-modulate experiments and spin–spin couplings in ^1H NMR of **1** allowed the identification of a β-D-glucuronopyranosyl (GlcA), a β-D-galactopyranosyl (Gal), a β-D-glucopyranosyl (Glc), a β-D-fucopyranosyl (Fuc), a α-L-rhamnopyranosyl (Rha), an α-L-arabinopyranosyl (Ara), and a β-D-xylopyranosyl (Xyl) moieties (Table 2). The β-anomeric configuration for the D-glucuronopyranosyl, D-galactopyranosyl, D-glucopyranosyl, D-fucopyranosyl, and D-xylopyranosyl moieties and the α-anomeric configuration for the arabinopyranosyl unit were determined by their large $^3J_{H1,H2}$ coupling constants of 7–8 Hz (Table 2). The β-D-xylopyranosyl unit, identified starting from anomeric signals at δ_H 4.70 (Xyl-H1) and δ_C 105.2 (Xyl-C1), the α-L-arabinopyranosyl unit (δ_H 4.59 and δ_C 104.8) and the β-D-galactopyranosyl unit (δ_H 4.84 and δ_C 103.7) were identified to be in terminal position, as observed by their ^{13}C NMR chemical shifts (Bock and Pedersen, 1983) (Table 2), suggesting most ramifications in the compound. The methyl doublet at δ_H 1.27 (*J* = 6.2 Hz) and the typical small doublet of anomeric proton at δ_H 5.18 (*J* = 1.1 Hz) were characteristic of a rhamnopyranosyl unit. It was identified as α anomeric configuration by the ^{13}C NMR chemical shift of its C-5 (δ_C 69.2) (Bock and Pedersen, 1983) and ROE effects observed between Rha H-1 and Rha H-2 and between Rha H-3 and Rha H-5. The deshielding of both Rha C-3 (δ_C 82.7) and Rha C-4 (δ_C 79.1) indicated a disubstitution of this rhamnopyranosyl moiety. The β-D-glucopyranosyl unit was identified starting from anomeric signals at δ_H 4.68 and δ_C 105.0, characterized by its large coupling constants up to 8 Hz. The deshielding of both proton H-3 (δ_H 4.25, *t*, *J* = 9.0 Hz) and carbon C-3 (δ_C 85.7) of this glucose suggested a substitution at this position. The β-D-fucopyranosyl unit was identified starting from the deshielded anomeric proton at δ_H 5.42 (*J* = 8.1 Hz) and characterized by its methyl doublet at δ_H 1.10 (*J* = 6.4 Hz) and its equatorial proton H-4 at δ_H 5.13 (*d*, *J* = 3.4 Hz). The deshielding of anomeric proton and the chemical shift of anomeric carbon at δ_C 95.3 suggested that this fucose was attached by an ester linkage to the C-28 carboxylic group of the aglycon. This was confirmed by the long-range correlation observed in the HMBC experiment between signals at δ_H 5.42 (Fuc H-1) and δ_C 178.2 (C-28 of gypsogenin). The fucose was substitued at position 2 as observed by its deshielded Fuc C-2 (δ_C 76.2). Starting from the anomeric proton at δ 4.49 (*d*, *J* = 7.5 Hz), a β-D-glucuronopyranosyl unit was identified with its carbonyl C-6 at δ 175.0. The deshielding of carbons C-2 and C-3 indicated a disubstitution of glucuronic acid (Table 2). Observation of long-range proton-carbon coupling in the HMBC spectrum between anomeric signal at δ_H 4.49 of the glucuronic acid and C-3 of gypsogenin, H-1 (δ_H 4.84) of the terminal galactose and C-2 (δ