Phytochemistry 152 (2018) 29–35

Contents lists available at ScienceDirect

Phytochemistry

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

Increased cytotoxic potential of infrequent triterpenoid saponins of *Cephalaria taurica* obtained through alkaline hydrolysis

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ARTICLE INFO

Article history:
Received 23 February 2018
Received in revised form
16 April 2018
Accepted 24 April 2018

Keywords:
Cephalaria taurica
Caprifoliaceae
Tauricosides A and B
Saponins
Cytotoxic activity

ABSTRACT

Phytochemical investigations of the aerial parts of the plant *Cephalaria taurica* Szabó. (Caprifoliaceae) have resulted in the isolation of nine oleanane-type triterpenoid saponins, of which two still remain undescribed. The structures of tauricosides A and B were characterized based on NMR analysis, HRESIMS spectrometry, and chemical evaluations. The saponins tauricosides A and B have been rarely reported in the literature due to the presence of eight sugar moieties, and this is also the first report of saponins containing eight sugar moieties in the Caprifoliaceae family.

The cytotoxic activities of tauricosides A and B, their undescribed prosapogenins, aglycone hederagenin, and *n*-butanol extract of *C. taurica* against the cancerous cells A-549, HeLa, PANC-1, and SH-SY5Y and the noncancerous HEK-293 cells were evaluated by the MTT method. Although tauricosides A and B and the crude *n*-butanol extract did not exhibit any activity at the tested concentrations on all the tested cells, after alkaline hydrolysis, the cytotoxic activity potential of the compounds was obviously improved. The most active compound, obtained after the alkaline hydrolysis of tauricoside B, showed a significant inhibitory effect, which was higher than that of the standard, commercially available drug doxorubicin, on the cancerous A-549, HeLa, PANC-1, and SH-SY5Y cells with IC_{50} values of 9.04, 8.75, 6.87, and 4.32 μM, respectively. In addition, prosapogenin, obtained after the alkaline hydrolysis of tauricoside A, exhibited considerable cytotoxic activity on the cancerous A-549, HeLa, PANC-1, and SH-SY5Y cells, with IC_{50} values of 13.19, 10.32, 11.91, and 7.49 μM, respectively. In conclusion, the alkaline hydrolysis of the saponins (tauricosides A and B) obviously improved their cytotoxic activity potential.

1. Introduction

The genus *Cephalaria* belonging to the Caprifoliaceae family, comprises about 94 species found in the Mediterranean region and the Middle East. There are 42 taxa in Turkey, of which 24 are endemic (Davis, 1972). This genus has been found to be a good source of active specialized metabolites such as saponins, which display a variety of bioactivities, including antimicrobial, cytotoxic, adjuvant, hemolytic, and immunomodulatory activities (Braca et al., 2004; Celenk et al., 2018; Kirmizigul et al., 1996; Podolak et al., 2010; Sarikahya et al., 2011; Top et al., 2017; Yang et al., 2005). Saponins have been traditionally used for several purposes such as for the preparation of soap, fish poison, and molluscicides and for industrial applications owing to their surface-active and foaming properties (Chen et al., 2010). In addition, recent scientific research has been primarily focusing on their health benefits such as cholesterol-lowering, anticancer, and adjuvant activities (Yang et al., 2005). Besides saponins, several flavonoids, alkaloids, lignans, iridoids, and their glycosides have been previously isolated from *Cephalaria* and their biological activities have also been reported (Aliev et al., 1975; Kayce and Kirmizigul, 2010; Movsumov et al., 2009; Mustafaeva et al., 2011, 2008; Pasi et al., 2002; Sarikahya et al., 2015).

In this study, we describe the isolation and the structural determination of nine triterpenoid saponins, two of which have not been previously described, namely, tauricosides A and B (**1** and **2**) isolated from the aerial parts of *Cephalaria taurica* Szabó. (Caprifoliaceae). The structures of the saponins were determined as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-xylopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**1**) and 3-*O*-α-L-

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https://doi.org/10.1016/j.phytochem.2018.04.015
0031-9422/

rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-[-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-]α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**2**). The structures of these compounds were identified using extensive spectroscopic techniques, along with 1D-, 2D-NMR, HRESIMS, and chemical methods, including silylation, and alkaline and acidic hydrolysis. Alkaline hydrolysis of the tauricosides A-B yielded two undescribed prosapogenins (Fig. 1). The cytotoxic activities of these two semi-synthetic saponins, obtained after alkaline hydrolysis, were tested by MTT assay. For this purpose, triterpenoid saponins (**1**–**2**), their prosapogenins (**1a**-**2a**), aglycone (hederagenin), and *n*-butanol extract of *C. taurica* were evaluated for their cytotoxicity potential against the cancerous cells A-549, HeLa, PANC-1, and SH-SY5Y and the noncancerous cells HEK-293, for the first time.

2. Results and discussion

In this study, a total of nine saponins were isolated and purified from *C. taurica*. The structures of these saponins were identified by FT-IR, 1D- and 2D-NMR, and HRESIMS methods and by chemical methods. Based on the NMR analyses of the two compounds tauricosides A-B (**1**–**2**) and seven known compounds, dipsacoside B (**3**) (Mukhamedziev et al., 1971), elmalienoside B (**4**) (Sarikahya and Kirmizigul, 2012a), isacoside (**5**) (Kayce and Kirmizigul, 2010), macranthodin A (**6**), macranthodin B (**7**) (Mao et al., 1993), gazipashoside A (**8**), and gazipashoside B (**9**) (Sarikahya and Kirmizigul, 2012b) were structurally determined. Saponins containing eight sugar moieties, such as tauricosides A and B, have been rarely reported in the literature. Thus, this is the first report of saponins with eight sugar moieties in the Caprifoliaceae family.

Tauricoside A (**1**) was purified as a light yellow, amorphous solid and its molecular formula was determined as $C_{76}H_{124}O_{40}$ by HRESIMS at *m/z* 1699.7576 $[M+Na]^+$ (calcd. for $C_{76}H_{124}O_{40}Na$, 1699.7561). The IR spectrum exhibited absorption bands at 1740, 1380, 1026 cm^{-1} corresponding carbonyl, double bond and etheric functionalities, respectively. The ^{1}H-NMR spectrum (Table 2) displayed six tertiary methyl signals at δ_H 0.54 (H-24), 0.84 (H-25), 0.65 (H-26), 1.05 (H-27), 0.84 (H-29), 0.83 (H-30), (each 3H, s), an olefinic proton signal at δ_H 5.13 (H-12, 1H, br s), an oxygen-bearing methine proton at δ_H 3.33 (H-3, 1H, m) and one primary alcoholic function at δ_H 3.07 and 3.32 (H-23, 2H, m). The ^{13}C-NMR (Table 1) and correlations in the HSQC spectra proved the presence of 76 carbon signals, of which 30 were assigned to the aglycone moiety, comprising six methyls, eleven methylenes (one oxygenated), five methines (one oxygenated and one double bond), and eight quaternary carbons (one carbonyl), and of which forty-six were assigned to the eight monosaccharide moieties. The carbon resonances in the ^{13}C-NMR spectrum, for tertiary six methyl carbons at δ_C 13.5 (C-24), 16.1 (C-25), 17.2 (C-26), 26.0 (C-27), 33.2 (C-29), 23.8 (C-30), two olefinic carbons at δ_C 122.1, 143.9, the oxygen-bearing methine carbon at δ_C 80.4 (C-3), and the primary alcoholic carbon at δ_C 62.8 suggested that compound **1** possessed hederagenin as a known aglycone. The literature findings also confirm the exact structure of aglycone (Sharma et al., 2013). Along with 1D-NMR analysis, 2D-NMR spectra (^{1}H-^{1}H COSY, TOCSY, HSQC and HMBC) data proved of the exact structure of **1**. Beside that the downfield shifts of C-3 (80.4) and C-28 (175.7) of the aglycone indicated that compound **1** is a bis-desmosidic saponin. In the sugar region of ^{1}H- and ^{13}C-NMR spectrum, eight anomeric proton signals were observed at δ_H 4.28 (d, J = 6.6 Hz, Ara), 5.08 (br s, Rha I), 4.36 (d, J = 7.2 Hz, Xyl), 4.98 (br s, Rha II), 4.29 (d, J = 6.6 Hz, Glc I), 4.24 (d, J = 6.6 Hz, Glc II), 5.19 (d, J = 8.4 Hz, Glc III), and 4.16 (d, J = 7.8 Hz, Glc IV) which were clearly correlated in HSQC spectrum with the corresponding carbon resonances at δ_C 103.7, 100.3, 104.8, 100.9, 103.4, 103.6, 94.5, and 103.3, respectively. The coupling constants exhibited the two β-glycosidic linkages for four glucose and one xylose units and two α-glycosidic linkages for two rhamnose and one arabinose units. GC-MS analysis after acidic hydrolysis and silylation procedure was also confirmed the exact structure of monosaccharide units (see Extraction and Isolation Section). Analysis of the HMBC and TOCSY spectra then supported the connectivity of these spin coupling fragments. The HMBC correlations between H-1 of arabinose at δ_H 4.28 and C-3 of aglycone at δ_C 80.4 and between H-1 of glucose III at δ_H 5.19 and carbonyl carbon at δ_C 175.7 indicated that the glycosidic chains were located at C-3 and C-28 of aglycone, respectively. The other HMBC correlations between H-1 of rhamnose I at δ_H 5.08 and C-2 of arabinose at δ_C 74.4, between H-1 of xylose at δ_H 4.36 and C-3 of rhamnose I at δ_C 82.2, between H-1 of rhamnose II at δ_H 4.98 and C-2 of xylose at δ_C 80.8, between H-1 of glucose I at δ_H 4.29 and C-3 of xylose at δ_C 79.9, between H-1 of glucose II at δ_H 4.24 and C-4 of glucose I at δ_C 78.9 and between H-1 of glucose IV at δ_H 4.16 and C-6 of glucose III at δ_C 68.2 proved all the linking points between sugar to sugar units. As it is known, TOCSY spectrum is extremely useful for identifying protons on sugar rings. This method contains the data presented in a correlation spectroscopy, but also includes useful additional knowledge about correlations seen between distant protons of sugar units. Some chemical methods were also used for supporting to structural determination studies. After the alkaline hydrolysis of **1**, ^{1}H-NMR and HRESIMS data of prosapogenin **1a** presented the correct molecular formula and confirmed the structure. All of the above evidence confirmed the structure of compound **1** as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-xylopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named tauricoside A.

Compound **2** was isolated as a light brown, amorphous powder and was assigned the molecular formula $C_{76}H_{124}O_{40}$, as a result of its molecular ion $[M + Na]^+$ peak at *m/z* 1699.7570 in the HRESIMS (calcd. for $C_{76}H_{124}O_{40}Na$, 1699.7561). While the ^{13}C- and ^{1}H-NMR data (Tables 1 and 2) suggested that compound **2** had the same aglycone, named hederagenin with compound **1** (Sharma et al., 2013), the linkage points of sugar units to each other in both two compounds are different. The ^{1}H-NMR spectrum showed eight anomeric protons at δ_H 4.29 (br s, Ara), 5.11 (br s, Rha I), 4.38 (d, J = 7.2 Hz, Xyl), 5.10 (br s, Rha II), 4.33 (d, J = 7.2 Hz, Glc I), 4.25 (d, J = 7.8 Hz, Glc II), 5.22 (d, J = 7.8 Hz, Glc III), and 4.19 (d, J = 8.4 Hz, Glc IV) attributed from HMQC and HMBC experiment to four glucose units, two rhamnose units, one arabinose and one xylose units, which were also supported by ^{13}C-NMR signals. The ^{13}C-NMR spectrum exhibited the anomeric carbons of the eight sugar units gave chemical shifts at δ_C 103.3, 99.9, 104.0, 99.8, 102.0, 103.4, 94.0, and 103.0. The coupling constants also confirm the presence of β-glycosidic linkages for four glucose and one xylose units, and α-glycosidic linkages for one arabinose and two rhamnose units. Moreover, acid hydrolysis of compound **2** gave D-glucose, L-arabinose, L-rhamnose, and D-xylose which were identified by GC-MS analysis using authentic samples for comparison. The HMBC spectrum showed long range H-C connectivity between anomeric proton signals and aglycone carbons at δ_H 4.29 (Ara) and δ_C 79.4 (C-3 of aglycone), and δ_H 5.22 (Glc III) and δ_C 175.2 (C-28 of aglycone) confirmed the linkage points of the sugar moieties to the aglycone. After alkaline hydrolysis, HRESIMS application also proved this linkage and the structure of prosapogenin **2a**. An exact determination of the sequence and linkages between carbohydrate moieties was also obtained from the HMBC and TOCSY spectra (see Sup. Info.). In HMBC spectrum, key correlation peaks between the protons and carbons were observed between at δ_H 5.11 (Rha I) and δ_C