Trends Phytochem. Res. 1(3) 2017 149-152

Trends in Phytochemical Research (TPR)

Journal Homepage: http://tpr.iau-shahrood.ac.ir

Original Research Article

A new lupane triterpene glycoside from *Euphorbia boissierana* Prokh.

Lili Gvazava[1], Nana Gorgaslidze[1], Markus Ganzera[2] and Alexander Skhirtladze[1,]✉

[1]TSMU Iovel Kutateladze Institute of Pharmacochemistry, 36 P. Sarajishvili st., 0159 Tbilisi, Georgia
[2]University of Innsbruck, Institute of Pharmacy, Pharmacognosy, Innrain 80-82, 6020 Innsbruck, Austria

ABSTRACT

A new lupane triterpene glycoside (**1**) was isolated from the MeOH extract of the aerial parts of *Euphorbia boissierana* Prokh. The structure of **1** was elucidated as 3-[(O-β-D-fucopyranosyl-(1→2)-β-D-glucopyranosyl)oxy] lup-20(29)-en-3β, 28-diol. Structure elucidation was accomplished through the extensive use of 1D- and 2D NMR experiments including ^{1}H-^{1}H (COSY) and ^{1}H-^{13}C (HSQC, HMBC) spectroscopy along with ESI-MS and HR-ESI-MS. This is the first report of the isolation of betulin glycoside from the genus *Euphorbia*.

ARTICLE HISTORY

Received: 14 June 2017
Revised: 25 July 2017
Accepted: 12 August 2017
ePublished: 08 September 2017

KEYWORDS

Euphorbia boissierana Prokh.
Euphorbiaceae
Triterpene glycoside
Lupane
Betulin

1. Introduction

There are more than 10,000 species in the Euphorbiaceae family (The Plant List, 2013). The *Euphorbia* genus is the largest one with diterpenoids and triterpenoids as characteristic secondary metabolites (Giner et al., 2000). *Euphorbia* plants displayed a wide range of biological activities including of anticancer, antitumor (Tanaka et al., 2000), anti-diabetic (Kpodar et al., 2015), anticoagulant (Aalikhanipour et al., 2016), antiproliferative (Cateni et al., 2010), antioxidant and cytotoxic (Aslanturk and Celik, 2013), and modulation of multidrug resistance (Vasas et al., 2012).

Euphorbia boissierana Prokh. is an endemic plant of the 45 species growing in Georgia. The decoction from very small quantities of *E. boissierana* leaves was used in folkloric medicine for colds of the respiratory tract (Kadaev, 1963). Our previous studies on the chemical compositions of *E. armena*, *E. boissierana*, *E. stricta* and *E. glareosa* led to isolation and characterization of flavonoids, the new hydrolyzed tannins glareins A, B, and C (Gvazava and Alaniya, 1997, 2000, 2002, 2005), stilbenes, cycloartane glycosides (Gvazava et al., 1993; Gvazava and Kikoladze, 2009a, 2009b, 2014). This paper deals with the structural determination of a new lupane-type triterpene glycoside isolated from *E. boissierana* based on the spectroscopic analysis, including various two-dimensional (2D) NMR spectroscopic techniques, and the results of hydrolytic cleavage.

2. Experimental

2.1. Apparatus

Optical rotation was recorded on a Perkin-Elmer 192 polarimeter. GC: Agilent 7850B; HP-5 capillary column (28 m×0.32 mm, i.d.); detection by FID; detector temp. 260 °C; column temp. 180 °C; carrier gas N_2; flow-rate 40 mL/min). IR spectrum was recorded on a Perkin-Elmer 1600 spectrometer. NMR experiments were performed on an Avance II 600 MHz spectrometer (BrukerBioSpinGmbH, Rheinstetten, Germany) equipped with a Bruker TXI probe head at 300 K. All 1D- and 2D NMR spectra were recorded in CD_3OD (99.95%, Euriso-Top), and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC and HMBC spectra. The NMR data were processed using MestRe-C UXNMR software (Santiago de Compostela,

✉ Corresponding author: Alexander Skhirtladze
Tel: +995-32 223 07 55; Fax: +995-32 253 14 94
E-mail address: aleksandre.skhirtladze@yahoo.com

Spain). High-resolution masses were recorded on a microOTOF-QII (Bruker) mass spectrometer in the positive electrospray ionization (ESI) mode. Exact mass calibration was performed on daily bases with the ESI-L low concentration tuning mix from Agilent (Santa Clara, USA). Column chromatography was performed on Diaion HP-20 material (Sigma-Aldrich) and silica gel (0.063-0.100 mm, Merck). TLC was performed on silica gel plates (Merck silica gel 60 F254), which were developed in the solvent system $CHCl_3$/MeOH/H_2O (26:14:3). All solvents for extraction and chromatographic separation were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.2. Plant material

The aerial parts of *E. boissierana* were collected in July 2015 in Kakheti region (Georgia). Samples of *E. boissierana* were identified by Dr. Jemal Aneli, Department of Pharmacobotany, Institute of Pharmacochemistry, Tbilisi, Georgia, and a respective herbarium specimen (No. 9034) was deposited in this department.

2.3. Extraction and isolation

500 g of powdered aerial parts of *E. boissierana* were extracted by shaking with 80% MeOH (2.5 L) for one hour once at room temperature and twice at 60 ºC. The collected extracts were dried under reduced pressure (62 g) and the concentrate was partitioned between hexane (8 g), *n*-BuOH (43 g) and H_2O (11 g). Part of the BuOH extract (10 g) was subjected to Diaion HP-20 column chromatography (50×4 cm) and eluted with a H_2O/MeOH gradient system (10:0 to 0:10) yielding 4 fractions (500 mL each) - 30% methanol (1.8 g), 50% methanol (3.7 g), 80% methanol (4.0 g) and 100% methanol (0.5 g). Part of the 80% methanol fraction (2.5 g) was then separated by CC on silica gel (100 g, 500×25 mm) material and eluted with the solvent system $CHCl_3$/MeOH/H_2O (26:14:3). This approach resulted in the isolation of individual triterpene glycoside (**1**) in the yield 35 mg.

3. Results and Discussion

3.1. Structure elucidation

The aerial parts of *E. boissierana* were extracted with 80% MeOH once at room temperature and twice at 60 ºC. The so obtained extract was partitioned between hexane, *n*-butanol and water. A part of the *n*-butanolic extract was passed through a porous-polymer polystyrene resin (Diaion HP-20) column, and the fraction eluting with 80% MeOH was subjected to silica gel column chromatography (CC) to afford one new triterpene glycoside (**1**) (Fig. 1).

Compound **1** was obtained as a white amorphous powder. The HR-ESI-TOFMS of 1 showed an accurate $[M+H]^+$ ion peak at *m/z* 751.6509 in accordance with the empirical molecular formula of $C_{42}H_{70}O_{11}$, which was supported by the ^{13}C NMR spectrum with a total of 42 signals and DEPT data. The ^1H-NMR spectrum of **1** showed signals for six triterpenoid methyl groups at δ 1.68, 1.02, 0.98, 0.96, 0.82, and 0.76 (each s), and an exomethylene group at δ 4.68 and 4.58 (each br s), which are characteristic of the lup-20(29)-en structure (Muhammad et al., 2017), as well as signals for two anomeric protons at δ 5.14 (d, J=7.9 Hz) and 4.48 (d, J=8.0 Hz). The three-proton doublet signal at δ 1.47 (J=6.4 Hz) indicated the presence of one deoxyhexopyranosyl unit in **1**. Acid hydrolysis of **1** with in aqueous MeOH (50%) containing conc. H_2SO_4 resulted in the production of an aglycone, identified as lup-20(29)-ene-3β, 28-diol (betulin) (Fomogne-Fodjo et al., 2017), as well as D-glucose and D-fucose, as the

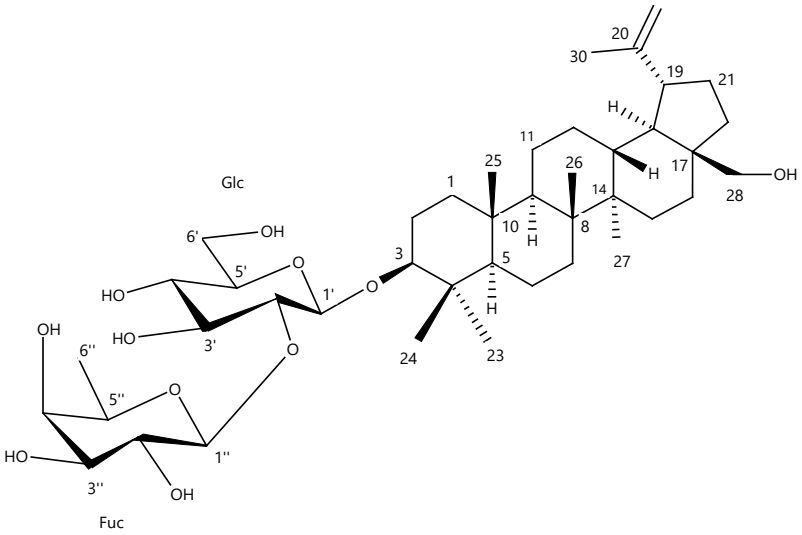

Fig. 1. Molecular structure of 3-[(O-β-D-fucopyranosyl-(1→2)-β-D-glucopyranosyl)oxy] lup-20(29)-en-3β, 28-diol as a new lupane triterpene glycoside (**1**).

carbohydrate components. In the ^{13}C-NMR spectrum of **1**, the C-3 and C-28 carbons of the aglycone moiety were observed at δ 91.0 and 61.8, respectively, which suggested that **1** was a 3-monodesmoside. The H-H COSY experiment with **1** allowed the sequential assignments of the signals from H-1 to H_2-6 and Me-6 of the monosaccharides. Their signal multiplet patterns and coupling constants (Table 1) indicated the presence of a β-D-glucopyranosyl unit (Glc) and a β-D-fucopyranosyl unit (Fuc). The proton resonances correlated with those of the one-bond coupled carbons using the HSQC spectrum. Comparison of the carbon chemical shifts thus assigned with those of the reference glycosides suggested that the Fuc group was presented as the terminal unit, whereas the Glc group was substituted at C-3. The anomeric configurations of the Glc and Fuc groups were ascertained by the relatively large *J* values of their anomeric protons (8.0 and 7.9 Hz). In the HMBC spectrum of **1**, long-range correlations were observed between H-1'' of Fuc at δ 5.14 and C-2' of glucose at δ 78.4 and between H-1' of Glc at δ 4.48 and C-3 of aglycone at δ 91.0. Thus, **1** was determined to be 3-[(O-β-D-fucopyranosyl-(1→2)-β-D-glucopyranosyl)oxy] lup-20(29)-en-3β, 28-diol.

3.2. 3-[(O-β-D-fucopyranosyl-(1→2)-β-D-glucopyranosyl)oxy] lup-20(29)-en-3β, 28-diol

White amorphous powder; $[\alpha]D^{25}$: -6.10 (*c* 0.8, MeOH). Rf=0.47 (solvent system: $CHCl_3$/MeOH/H_2O (26:14:3); IR ν_{max} (KBr) 3614, 3394, 2942, 1651, 1396 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1. HR-ESI-TOF-MS: 751.6509 ($[M+H]^+$, $C_{42}H_{71}O_{11}^+$; calcd. for 751.6516).

3.3. Acid hydrolysis

Compound **1** (25 mg) was dissolved in aqueous MeOH (50%, 10 mL) containing conc. H_2SO_4 (0.4 mL), refluxed for 8 h, cooled, and diluted with H_2O. The resulting precipitate was filtered off and purified by recrystallization from MeOH to afford white crystals (11 mg) of the aglycon ($C_{30}H_{50}O_2$). The aglycon was identified as lup-20(29)-ene-3β,28- diol (betulin) by comparing the NMR data with the literature (Fomogne-Fodjo et al., 2017). The aqueous layer was neutralized with dilute NaOH and concentrated to dryness. The resultant residue was dissolved in pyridine (1 mL), then $(CH_3)_3SiNHSi(CH_3)_3$ (1 mL) was added. After 10 min. at room temperature, the solution was blown to dryness under a stream of nitrogen. The residue was dissolved in diethyl ether and then subjected to chiral GC-MS analysis. Absolute configurations of monosaccharides were found to be D-glucose and D-fucose by comparison of the retention times of their derivatives with those of D-fucose (7.04 min.) and D-glucose (10.53 min.) derivatives prepared in the same way.

Table 1

^{1}H- and ^{13}C-NMR (600 MHz, CD_3OD) Data of the compound **1** (*J* in Hz, δ in ppm).

C	δ(C)	δ(H)
1	40.1	1.65 (*m*), 0.90 (*m*)
2	27.6	1.60 (*m*), 1.00 (*m*)
3	91.0	3.18 (*dd*, *J*=11.7, 4.7)
4	40.4	-
5	56.5	0.68 (*d*, *J*=10.0)
6	19.6	1.52 (*m*), 1.39 (*m*)
7	35.5	1.39 (2H) (*m*)
8	42.2	-
9	51.7	1.28 (*m*)
10	38.6	-
11	22.1	1.40 (*m*), 1.20 (*m*)
12	26.4	1.63 (*m*), 1.04 (*m*)
13	38.3	1.64 (*m*)
14	44.0	-
15	28.2	1.70 (*m*), 1.05 (*m*)
16	30.3	1.92 (*m*), 1.20 (*m*)
17	49.1	-
18	50.0	1.58(*m*)
19	49.0	2.38 (*m*)
20	151.7	-
21	31.0	1.96 (*m*), 1.40 (*m*)
22	35.2	1.85 (*m*), 1.03 (*m*)
23	29.3	0.96 (*s*)
24	16.6	0.76 (*s*)
25	17.3	0.82 (*s*)
26	17.2	1.02 (*s*)
27	15.9	0.98 (*s*)
28	61.8	3.79, (*d*, *J*=10.8), 3.33 (*d*, *J*=10.8)
29	110.7	4.68 (*br s*), 4.58 (*br s*)
30	20.3	1.68 (*s*)
Glc		
1'	100.2	4.48 (*d*, *J*=8.0)
2'	78.4	3.36 (*dd*, J=9.0, 8.0)
3'	79.1	3.46 (*dd*, *J*=9.0, 9.0)
4'	71.5	3.29 (*dd*, *J*=9.0, 9.0)
5'	77.8	3.26 (*ddd*, *J*=9.0, 4.5, 2.0)
6'	62.5	3.86 (*dd*, *J*=12.0, 2.0)
		3.67 (*dd*, *J*=12.0, 4.5)
Fuc		
1''	106.4	5.14 (*d*, *J*=7.9)
2''	73.1	4.42 (*dd*, *J*=9.7, 7.9)
3''	75.4	4.06 (*dd*, *J*=9.7, 3.2)
4''	72.8	3.97 (*dd*, *J*=3.2, 3.0)
5''	71.4	3.77 (*qd*, *J*=6.4, 3.0)
6''	17.2	1.47 (*d*, *J*=6.4)

4. Concluding remarks

From the BuOH extract of aerial parts of Georgian endemic plant *E. boissierana* the major compound (**1**) has been isolated using Diaion HP-20 and silica gel column chromatography. The structure of **1** was elucidated as 3-[(O-β-D-fucopyranosyl-(1→2)-β-D-glucopyranosyl) oxy] lup-20(29)-en-3β, 28-diol. Structure elucidation was accomplished through the acid hydrolysis, physical and chemical data and the extensive use of 1D- and

2D NMR experiments including ^{1}H-^{1}H (COSY) and ^{1}H-^{13}C (HSQC, HMBC) spectroscopy along with ESI-MS and HR-ESI-MS, as well as GC-MS. Compound **1** is a new lupane-type triterpene glycoside with two monosaccharides. The structure of the aglycone moiety is lup-20(29)-ene-3β, 28-diol (betulin). This is the first report of the isolation of betulin glycoside from the genus Euphorbia. Betulin glycosides are mainly synthesized (Korda et al., 2017) and have been isolated only from *Oplopanax elatus* Nakai (Araliaceae) (Wang and Xu, 1993) and from *Stryphnodendron fissuratum* Mart. (Leguminosae) (Yokosuka et al., 2011).

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors thanks Simon Moosmang and PhD student Vazha Nebieridze for providing them NMR spectra.

References

Aalikhanipour, M., Sardari, S., Eslamifar, A., Rezvani, M., Azhar, A., Nazari, M., 2016. Evaluating the anticoagulant effect of medicinal plants *in vitro* by cheminformatics methods. J. Herb. Med. 6(3), 128-136.

Aslanturk, O.S., Celik, T.A., 2013. Antioxidant, cytotoxic and apoptotic activities of extracts from medicinal plant *Euphorbia platyphyllos* L. J. Med. Plants Res. 7(19), 1293-1304.

Cateni, F., Zilic, J., Zacchigna, M., Procida, G., 2010. Cerebrosides with antiproliferative activity from *Euphorbia peplis* L. Fitoterapia 81(2), 97-103.

Giner, J.L., Berkowitz, J.D., Andersson, T., 2000. Nonpolar components of the latex of *Euphorbia peplus*. J. Nat. Prod. 63(2), 267-269.

Gvazava, L.N., Alaniya, M.D., 1997. Flavonoids of *Euphorbia armena*. Chem. Nat. Compd. 33(2), 210.

Gvazava, L.N., Alaniya, M.D., 2000. Hydrolyzed tannins of *Euphorbia glareosa* leaves. Structure of glarein A. Chem. Nat. Compd. 36(2), 140-143.

Gvazava, L.N., Alaniya, M.D., 2002. Hydrolyzed tannins of *Euphorbia glareosa* leaves. Structure of glarein C. Chem. Nat. Compd. 38(4), 323-325.

Gvazava, L.N., Alaniya, M.D., 2005. Hydrolyzed tannin from *Euphorbia glareosa* leaves. Chem. Nat. Compd. 41(3), 308-310.

Gvazava, L.N., Kikoladze, V.S., 2009a. Stilbene glycosides from *Euphorbia armena* roots. Chem. Nat. Compd. 45(6), 859-860.

Gvazava, L.N., Kikoladze, V.S., 2009b. Cycloartane glycosides from *Euphorbia glareosa*. Chem. Nat. Compd. 45(4), 595-596.

Gvazava, L.N., Kikoladze, V.S., 2014. Cycloartane glycosides from *Euphorbia boissierana*. Chem. Nat. Compd. 50(6), 1012-1015.

Gvazava, L.N., Alaniya, M.D., Kikoladze, V.S., Kemertelidze, E.P., 1993. Triterpene glycoside from Euphorbia stricta. Proc. Geo. Nat. Acad. Sci. 19(3-4), 334-336.

Kadaev, G.N., 1963. Medicinal plants of Karachay-Cherkessia. Cherkessk, 288 p. (in Russian).

Korda, A., Pakulski, Z., Cmoch, P., Gwardiak, K., Karczewski, R., 2017. Sugar migration induced by the Wagner-Meerwein rearrangement of 28-*O*-glycosyl-betulin derivatives. Tetrahedron 73(13), 1740-1744.

Kpodar, M.S., Lawson-Evi, P., Bakoma, B., Eklu-Gadegbeku, K., Agbonon, A., Aklikokou, K., Gbeassor, M., 2015. Ethnopharmacological survey of plants used in the treatment of diabetes mellitus in south of Togo (Maritime Region). J. Herb. Med. 5(3), 147-152.

Muhammad, D., Lalun, N., Bobichon, H., Debar, E.L.M., Gangloff, S.C., Nour, M., Voutquenne-Nazabadioko, L., 2017. Triterpenoid saponins and other glycosides from the stems and bark of *Jaffrea xerocarpa* and their biological activity. Phytochemistry 141(1), 121-130.

Fomogne-Fodjo, M.C.Y., Ndinteh, D.T., Olivier, D.K., Kempgens, P., van Vuuren, S., Krause, R.W.M., 2017. Secondary metabolites from *Tetracera potatoria* stem bark with antimycobacterial activity. J. Ethnopharmacol. 195(1), 238-245.

Tanaka, R., Kasubuchi, K., Kita, S., Tokuda, H., Nishino, H., Matsunaga, S., 2000. Bioactive steroids from the whole herb of *Euphorbia chamaesyce*. J. Nat. Prod. 63(1), 99-103.

The Plant List, 2013. Version 1.1. Published on the Internet. http://www.theplantlist.org/.

Vasas, A., Sulyok, E., Martins, A., Re´dei, D., Forgo, P., Kele, Z., Zupko, I., Molnar, J., Pinke, G., Hohmann, J., 2012. Cyclomyrsinane and premyrsinane diterpenes from *Euphorbia falcata* modulate resistance of cancer cells to doxorubicin. Tetrahedron 68(4), 1280-1285.

Yokosuka, A., Kawakami, S., Haraguchi, M., Mimaki, Y., 2011. Seven new triterpene glycosides from the pericarps of *Stryphnodendron fissuratum*. Phytochem. Lett. 4(3), 259-266.

Wang, G., Xu, J., 1993. Chemical study of glycosides of *Oplopanax elatus* Nakai. Zhongguo Yaoxue Zazhi (Beijing, China), 28(10), 593-594.