

Poly[3-(3,4-Dihydroxyphenyl)Glyceric Acid] from *Cynoglossum officinale* L. (Boraginaceae)

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According to UV, IR, ^1H NMR data and to a 2D heteronuclear $^1\text{H}/^{13}\text{C}$ HSQC experiment the main chemical constituent of water-soluble high-molecular preparation of *Cynoglossum officinale* (Boraginaceae) (HMP-CO) by analogy with *S. asperum*, *S. caucasicum*, *S. officinale*, *S. grandiflorum* and *Anchusa italica* high-molecular fractions (HMFs), was found to be a caffeic acid-derived polymer, namely poly[oxy-1-carboxy-2-(3,4-dihydroxy-phenyl)ethylene] or poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA). The IR spectrum of HMP-CO showed bands characteristic of phenols, carboxylic acids and ether groups. The ^1H NMR spectrum of HMP-CO showed signals at 5.18 and 4.65 ppm, assigned to H-1 and H-2, respectively. A ^1H signal at 7.15 ppm was assigned to the aromatic proton H-2'' and a signal at 7.04 ppm to the aromatic protons H-5'' and H-6''. These assignments were confirmed by a 2D heteronuclear $^1\text{H}/^{13}\text{C}$ HSQC experiment. © 2020 Bull. Georg. Natl. Acad. Sci.

Caffeic acid-derived polymer, poly[3-(3,4-dihydroxyphenyl)glyceric acid], poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene], *Cynoglossum officinale*

Many plants of the Boraginaceae family have medicinal properties and they are used in traditional medicines in several countries for treating wounds, skin diseases, fever, chest pains etc. [1,2]. In our previous studies we isolated high molecular fractions (HMFs) from *S. asperum*, *S. caucasicum*, *S. Officinale*, *S. grandiflorum* and *Anchusa italica*, all of them of the Boraginaceae family [3-8]. We found that their main chemical constituent was a regular caffeic acid-derived polymer, namely poly- [oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] or poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA) (Fig.) [5-8].

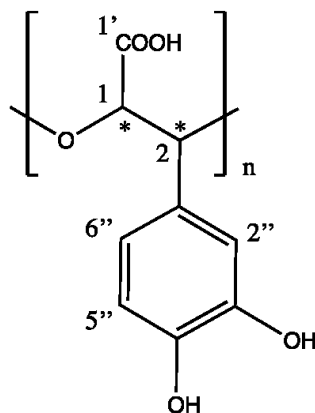


Fig. Poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] or PDPGA.

The repeating unit of this polymer, namely the 3-(3,4-dihydroxyphenyl)glyceric acid residue (Fig.) contains two aliphatic chiral carbon atoms C1 and C2. As the profiles of the circular dichroism (CD) spectra of **PDPGA** did not coincide with those of the synthetic enantiomers of the **PDPGA** monomers (+)-(2*R*,3*S*) and (–)-(2*S*,3*R*)-2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid we excluded the (1*R*,2*S*) and (1*S*,2*R*) configurations for chiral atoms C1 and C2 in the repeating unit of **PDPGA**. Hence, we supposed that C1 and C2 atoms have either (1*R*,2*R*) or (1*S*,2*S*) configurations and consequently the denomination of **PDPGA** should be either poly[oxy-(1*R*)-1-carboxy-(2*R*)-2-(3,4-dihydroxyphenyl)ethylene] or poly[oxy-(1*S*)-1-carboxy-(2*S*)-2-(3,4-dihydroxyphenyl)ethylene] [9]. The specific rotation of **PDPGA** is $[\alpha]_{\text{D}}^{20^\circ\text{C}} = +129.92^\circ$ ($c = 1.0$, H_2O). However, as there is no correlation between an absolute stereochemical configuration and the specific rotation, we expect to settle this point by resorting to infrared vibrational circular dichroism (VCD) [10] or to computer modeling using optical rotation values obtained from density functional theory (DFT) calculations [11]. Consequently, the determination of the exact absolute configuration of these C atoms is a topic for further research.

PDPGA is interesting from a pharmaceutical point of view, because its anticomplementary, antioxidant and anti-inflammatory activity [4,12–14] and wound-healing properties [15,16]. However, its most important pharmacological property is its anticancer activity against prostate cancer cells *in vitro* and *in vivo* experiments [17].

The aim of the present research was to detect **PDPGA** in high molecular preparations (**HMP**) of *Cynoglossum officinale* L. (**HMP-CO**) hound's tongue, a species of another genus of the Boraginaceae family. A literature survey revealed that very little phytochemical studies have been carried out in *Cynoglossum* genus and that they are known to accumulate pyrrolizidine alkaloids as a major means of chemical self-defense [18].

Materials and Methods

Apparatus: The UV spectrum was recorded on a UV/VIS spectrophotometer (Mecasys Optizen Pop, Mecasys Co., Ltd., Daejeon, Korea). The IR spectrum in KBr disc was obtained on a FT-IR spectrophotometer (Agilent Resolutions Pro, Agilent Technologies, Inc. Santa Clara, CA, USA). A Varian NMR System (Palo Alto, CA, USA), fitted with a $\text{CHX}^1\text{H}/^{13}\text{C}/^{15}\text{N}-^{31}\text{P}$ probe head, gradient module and variable temperature unit was used for all NMR experiments. The spectrometer resonance frequency for ^1H was 499.61 MHz. Sample of **HMP-CO** was prepared from ca. 16 mg in 1.0 mL D_2O . TSP-Na (sodium 3-trimethylsilyl-2,2,3,3-tetradeutero propionate, ca. 1.0 mg) was added as the internal reference ($\delta\ ^1\text{H} = 0.00$ ppm, $\delta\ ^{13}\text{C} = -2$ ppm). The ^1H NMR and $^1\text{H}/^{13}\text{C}$ HSQC spectra of **HMP-CO** were run at 50°C. All NMR spectra were processed with the Mestre NOVA software (version 10.0.2, Mestrelab Research, S. L., Santiago de Compostela, Spain).

Plant material: Fresh stems of *C. officinale* were collected on 19.07.2016 from their natural habitat near the town of Gori, Georgia. A voucher specimen was deposited at the Tbilisi State Medical University I. Kutateladze Institute of Pharmacochimistry.

Extraction and isolation: Ground stems of *C. officinale* was air-dried and pretreated in a Soxhlet apparatus with chloroform, methanol and acetone, sequentially. After this treatment of 25.04 g of the obtained materials was extracted with hot water followed by dialysis [3] 3.11 g of a water-soluble crude polysaccharide preparation of *C. officinale* (**CPP-CO**) was obtained. Based on dry biomass the yield was 12.4%. Further fractionation procedure of 0.730 g of **CPP-CO** by ultrafiltration on a membrane filter with a cut off value of 500 kDa [7] yielded 0.120 g of high-molecular (>500 kDa) preparation of *C. officinale* (**HMP-CO**) based on **CPP-CO** 16.43% and dry biomass 1.04%, respectively.

Carbohydrate analysis of CPP-CO and HMP-CO: the total sugars contents were determined spectrophotometrically by the phenol-conc. H_2SO_4 reaction (**CPP-CO** – 30%, **HMP-CO** – 17%) [19], the fructose by resorcinol-HCl reagent (**CPP-CO** – 2.2%, **HMP-CO** – 2.1%) [20], the glucose by glucosooxidase reagent (**CPP-CO** – 1.55%, **HMP-CO** – 0.67%) [21] and the galacturonic acid with the *m*-hydroxydiphenyl- H_2SO_4 reagent (**CPP-CO** – 12%, **HMP-CO** – 6.37%) [22], respectively. Besides galactose, mannose, arabinose, xylose and rhamnose were analyzed by TLC and GC after hydrolysis as described in our previous paper [10].

HMP-CO: UV spectrum (H_2O , λ_{max} , nm): 216, 236 (shoulder), 282 (shoulder), 286.

IR spectrum (KBr, ν , cm^{-1}): 3415 (OH); 2928 (CH); 1604 (ionized carboxylate); 1511, 1445 (aromatic C=C); 1410, 1219 (phenols); 1266, 1123, 1076, 1047 (R-O-R'); 870 (C-H in the aromatic ring with one isolated hydrogen atom); 821 (C-H in the aromatic ring with two neighboring hydrogen atoms).

Results and Discussion

The scheme of isolation and purification procedures of **HMP-CO** allowed us to remove completely hepatotoxic pyrrolizidine alkaloids [23] and most ballast polysaccharides. However, and by direct comparison to **HMFs** of *Symphytum* and *Anchusa* [4-8], we concluded that **HMP-CO** contained some residual polysaccharides.

The UV spectrum (see materials and methods) of water-soluble **HMP-CO** showed the absorption maxima indicating a presence of polymer of phenolic character and was identical to UV spectra of polyethers obtained from *S. asperum*, *S. caucasicum*, *S. officinale*, *S. grandiflorum* and *A. italica* [5-8].

The IR spectrum (see materials and methods) of **HMP-CO** showed absorption bands characteristic of phenol-carboxylic acids. Absorption bands corresponding to the hydroxyl groups

attached to the aromatic ring, as well as the carboxyl and ether groups were observed. Again, the IR spectrum of **HMP-CO** was very similar to that of phenolic polymers from *S. asperum*, *S. caucasicum*, *S. Offi-cinale*, *S. grandiflorum* and *A. italica* [5-8].

Then we tried to detect of **PDPGA** in **HMP-CO** using different techniques of NMR spectroscopy. The two signals in the ^1H NMR spectrum of **HMP-CO** with chemical shifts of 5.18 and 4.65 ppm obviously belonged to H-1 and H-2 (Fig. 1), respectively, linked with oxygen-bound aliphatic carbon atoms C1 and C2 (Fig.). The signal with chemical shift 7.15 ppm was assigned to aromatic proton H-2" (Fig. 1) and that at 7.04 ppm with an intensity twice as much as that at 7.15 ppm was assigned to H-5" and H-6" (Fig.) [5-8].

Unfortunately we could not quite get rid of all polysaccharides by ultrafiltration so our **HMP-CO** preparations still contained residual polysaccharides. Some broad resonances in the ^1H NMR spectrum (δ_{H} 3.7 – 4 ppm) were associated to carbohydrates impurities.

The $^1\text{H}/^{13}\text{C}$ HSQC spectrum showed a cross peak at 77.5 ppm (^{13}C) with 5.2 ppm (^1H), and another at 80 ppm (^{13}C) with 4.7 ppm (^1H), which were consistent with the aliphatic CH-1 and CH-2, respectively, of **PDPGA** [5-8]. Other cross peaks were also observed in the $^1\text{H}/^{13}\text{C}$ HSQC spectrum, namely correlations between the following aromatic proton and carbon atom pairs: δ 7.16/117, 7.05/118, 7.03/122.5 ppm, which were respectively assigned to CH-2", CH-5" and CH-6" (Fig. 1) [5-8]. A ^{13}C NMR experiment of **HMP-CO** was unsuccessful due to low sensitivity, but the correlations in the 2D $^1\text{H}/^{13}\text{C}$ HSQC spectrum unambiguously corroborated the structure of poly-[3-(3,4-dihydroxyphenyl)glyceric acid] (**PDPGA**) [Fig. 1] [5-8]. Thus, **PDPGA** is the main chemical constituent of **HMP-CO** by analogy of **HMFs** from *S. asperum*, *S. caucasicum*, *S. officinale*, *S. Grandiflorum* and *A. italica* [5-8].

The detection of **PDPGA** in the genus *Cynoglossum* shows that its biosynthesis is not a unique feature of the genera *Symphytum* and *Anchusa*. The observation of this compound in the stems of *C. officinale* similarly to *Symphytum* species [8,24] is important from economic viewpoint

because the isolation of biologically active compounds from vegetative plant parts is simple and cheap. Besides, the presence of **PDPGA** in different Boraginaceae genera expands the source of raw materials of this biologically active polymer.

ფარმაკოქიმია

Cynoglossum officinale L.-ის (Boraginaceae)

პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავა]

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უი, იწ, ^1H ბმრ, 2D ჰეტერობირთვული $^1\text{H}/^{13}\text{C}$ HSQC ექსპერიმენტების მონაცემების საფუძველზე დადგინდა, რომ მსგავსად *Symphytum asperum*, *S. caucasicum*, *S. officinale*, *S. grandiflorum* და *Anchusa italica*-ს მაღალმოლეკულური ფრაქციებისა *Cynoglossum officinale* L.-ის (Boraginaceae) წყალში ხსნადი მაღალმოლეკულური პრეპარატის (მმპ-CO) ძირითადი კომპონენტია კოფეინის მჟავას წარმოებულის პოლიმერი, კერძოდ, პოლი[ოქსი-1-კარბოქსი-2-(3,4-დიჰიდროქსიფენილ)ეთილენი], ანუ პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავა] (პდფგმ).CO -მმპ-ის იწ-მა სპექტრმა აჩვენა ფენოლ-კარბონმჟავებისათვის დამახასიათებელი შთანთქმის ზოლები, კერძოდ, არომატულ რგოლთან მიერთებული ჰიდროქსილის, აგრეთვე კარბოქსილის და ეთერის ჯგუფების შესაბამისი შთანთქმის ზოლები. CO-მმპ-ის ^1H ბმრ-ის სპექტრის სიგნალები ქიმიური ძვრადობებით 5,18 და 4,65 მმნ მიეკუთვნება პდფგმ-ის ჟანგბადთან ბმულ ალიფატური ნახშირბადის ატომებთან დაკავშირებულ H-1 და H-2. სიგნალი ქიმიური ძვრადობით 7,15 მმნ მიეკუთვნება არომატულ პროტონს H-2" და ორმაგი ინტენსივობის სიგნალი ქიმიური ძვრადობით 7,04 მმნ მიეკუთვნება არომატულ პროტონებს H-5" და H-6". CO-მმპ-ის 2D ჰეტერობირთვული $^1\text{H}/^{13}\text{C}$ HSQC ექსპერიმენტის მონაცემების საფუძველზე დადგინდა კორელაცია ალიფატურ პროტონებსა და ნახშირბადის ატომებს შორის ქიმიური ძვრადობებით δ 5,2/77,5, 4,7/80 მმნ, რომლებიც შესაბამისობაშია პდფგმ-ის CH-1 და CH-2. CO-მმპ-ის 2D ჰეტერობირთვული $^1\text{H}/^{13}\text{C}$

HSQC ექსპერიმენტის მონაცემების მიხედვით დადგინდა აგრეთვე კორელაცია არომატულ პროტონებსა და ნახშირბადის ატომებს შორის ქიმიური ძვრადობებით δ 7,16/117, 7,05/118, 7,03/122,5 მმნ, რომლებიც შეესაბამება პდფგმ-ის CH-2", CH-5" და CH-6".

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- via targeting androgen receptor, cell cycle arrest and apoptosis. *Carcinogenesis*, **33**, 8: 1572–1580. PMID: 22693258, PMCID: PMC3499058, <https://doi.org/10.1093/carcin/bgs202>.
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