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Pharmacochemistry

Carbohydrate-Based Biopolymers: Biologically Active Poly[3-(3,4-Dihydroxyphenyl)Glyceric Acid] from *Borago officinalis*

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A high-molecular water-soluble preparation from stems of Borago officinalis (Boraginaceae family) was isolated. According to data from UV, IR, ^{1}H, ^{13}C NMR, gCOSY and 2D heteronuclear ^{1}H/^{13}C gHSQCED experiments, the main chemical constituent of this water-soluble high-molecular preparation from stems of *Borago officinalis* (HMP-BS) was found to be a biologically active caffeic acid-derived polymer, namely poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] also referred to as poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA). PDPGA was previously detected in high-molecular preparations of *Symphytum asperum, S. caucasicum, S. grandiflorum, Anchusa italica* and *Cynoglossum officinale*. The detection of this compound in different genera of the Boraginaceae family is interesting as this unusual caffeic acid-derived polymer could be considered as chemotaxonomic marker among Boraginaceae plants. Thus, PDPGA is interesting due to the importance of its chemotaxonomic significance, the potential biomedical applications of the Boraginaceae plants and the chemical importance of PDPGA. The presence of poly[3-(3,4-dihydroxyphenyl)glyceric acid] in multiple Boraginaceae species expands the resources of raw materials for this biologically active polymer. © *2021 Bull. Georg. Natl. Acad. Sci.*

Carbohydrate-based biopolymers, caffeic acid-derived polyether, poly[3-(3,4-dihydroxyphenyl)glyceric acid], poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene], *Borago officinalis*

In previous publications we reported that the main chemical constituent of high-molecular water-soluble preparations from *Symphytum asperum, S. caucasicum, S. officinale, S. grandiflorum, Anchusa italica* and *Cynoglossum officinale* (Boraginaceae) was biologically active caffeic acid-derived polymer, namely poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] that is poly [3-(3,4-dihydroxyphenyl)glyceric acid] (**PDPGA**) [1-5]. The polyoxyethylene chain is the backbone of this

polymer molecule with 3-(3,4-dihydroxyphenyl)glyceric acid residue as the repeating unit (Fig. 1). This compound represents a class of natural polyethers. Within the field of pharmacologically active biopolymers the area of stable polyethers seems rather new and attractive. **PDPGA** as a unique natural polyether contains aliphatic ether groups in its polymer backbone. Ether bonds are found in a wide variety of natural products – mainly secondary metabolites, or in aromatic polymer such as lignin. Lignin contains ether links between two aromatic rings or between an aromatic ring and an aliphatic moiety [6]. However, reports concerning polymers that contain aliphatic ethers as repeating unit are missing. On the other hand **PDPGA** as a 3,4-dihydroxyphenyl derivative of poly(2,3-glyceric acid ether) also belongs to a rare class of carbohydrate-based biopolymers, namely poly(sugar acids). Its basic monomeric moiety, glyceric acid, is a natural three-carbon sugar acid which is an oxidative form of the simplest of all common aldoses, namely glyceraldehyde. In this case poly(2,3-glyceric acid ether) chain constitutes the backbone of this polymeric molecule with 3,4-dihydroxyphenyl groups as regular substituents at the 3C positions in the chain. Every repeating structural unit of **PDPGA** contains three reactive functional groups, two *ortho*-related phenolic hydroxyl groups and one carboxyl group. Consequently, the polymeric molecule of **PDPGA** bears many of these functional groups along the polymeric chain. This multifunctionality justifies that **PDPGA** belongs to several important classes of biopolymers. Moreover, multifunctionality should be a reason of its wide spectrum of biological activities, as **PDPGA** is endowed with intriguing *in vitro* and *in vivo* pharmacological properties: anti-inflammatory, antioxidant, anticomplementary and anticancer [7-9]. Medicinal effects of *Symphytum*, *Anchusa* and *Cynoglossum* plants used in folk medicine could be attributed to this polymer, rendering **PDPGA** a unique candidate for many biomedical applications.

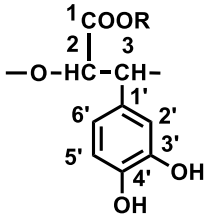
Results and Discussion

Within our ongoing search for biologically active caffeic acid-derived polymers in plant species belonging to different genera of the Boraginaceae family, we have carried out the isolation and structure elucidation of the main chemical constituent of water-soluble high-molecular preparation from *Borago officinalis* stems **(HMP-BS)**. The high-molecular (>500 kDa) preparation **HMP-BS** was isolated from the crude polysaccharides by means of ultrafiltration using membrane filters with a cut-off value of 500 kDa, as described in some of our earlier publications [1-5]. In our previous studies we found that the crude materials obtained from *Symphytum*, *Anchusa* and *Cynoglossum* plants essentially consist of mixtures of biologically active **PDPGA** and then biologically inactive polysaccharides, mainly glucofructan and pectic acid [1-5]. That is why during the ultrafiltration process to monitor the removal of biologically inactive polysaccharides we identified fructose and galacturonic acid as the main constituents of glucofructan and pectic acid, respectively.

The UV spectrum (see materials and methods) of **HMP-BS** showed absorption maxima at 214, 286 nm identical to the UV spectrum of polyether **PDPGA** [1-5]. The IR spectrum of **HMP-BS** showed some absorption bands characteristic of phenols and carboxylic acids. The IR spectrum of **HMP-BS** was also very similar to that of phenolic polyether **PDPGA** [1-5]. Thus, the presence of **PDPGA** in **HMP-BS** was supposed on the basis of its UV and IR spectral data.

Then we tried to identify **PDPGA** in **HMP-BS** by using different techniques of NMR spectroscopy, namely ^{1}H and ^{13}C NMR, gCOSY and 2D heteronuclear $^{1}H/^{13}C$ gHSQCED. The assignment of the complete set of resonances signals for **PDPGA** in the ^{13}C NMR and ^{1}H NMR spectra, based on correlations between protons and carbon atoms by means of the 2D $^{1}H/^{13}C$ gHSQCED spectra, was carried out as described in the previous papers [1-5] and are listed in Table 1.

Table. The signal assignment in the ^{13}C and ^{1}H NMR spectra of PDPGA from *B.officinalis* stems

	C atom no.	^{13}C chemical shift, δ_C, ppm	^{1}H chemical shift, δ_H, ppm
 **Fig. 1**. The repeating unit of PDPGA; R=H, CH_3.	1	175.00 (COO) 172.00 ($COOCH_3$) 53.45 (OCH_3)	 3.8(OCH_3)
	2	77.48	5.16
	3	79.56	4.67
	1'	130.71	
	2'	116.61	7.16
	3'	143.89	
	4'	143.00	
	5'	117.82	7.06
	6'	121.48	7.06

The gCOSY spectrum showed a cross peak between the signals at 5.16 and 4.67 ppm, which was consistent with the coupling between H-2 and H-3 of **PDPGA** (Table 1, Fig. 1).

We found that the main chemical constituent of **HMP-BS** was **PDPGA.** However we could not quite get rid of all common polysaccharides and some resonances in the ^{1}H NMR spectra that were associated to carbohydrates impurities which, apparently, are not covalently bound to **PDPGA**. In the ^{1}H NMR, and ^{13}C NMR spectra of **HMP-BS**, in addition to the signals of **PDPGA** (Table), other resonances of rhamnogalacturonan of pectic nature were also seen [10]. The presence of polysaccharides impurities in **HMP-BS** would imply similar (same order of magnitude) molecular weights for **PDPGA** and residual polysaccharides. Besides, **PDPGA** molecules can form complex macromolecular aggregates with these residual polysaccharides upon hydrogen bonds formation. This would explain why it proved not feasible to separate **PDPGA** from residual polysaccharides by ultrafiltration and gel-filtration chromatography [1-5,11].

Conclusion

Thus, according to UV, IR and NMR data, the main chemical component of water-soluble high-molecular preparation from *B.officinalis* stems **(HMP-BS)**, similarly to *Symphytum*, *Anchusa* and *Cynoglossum* plants, was found to be a representative of regular carbohydrate-based biopolyethers, namely poly[3-(3,4-dihydroxy-phenyl)glyceric acid] (**PDPGA**) (Fig. 1) [1-5]. Similarly to *A. italica* and *S. grandiflorum* biopolymers, most of the carboxylic groups of this caffeic acid-derived polymer of *B. officinalis* are methylated. The detection of **PDPGA** in the genus *Borago* shows that its biosynthesis is a unique feature not only for the following genera *Symphytum*, *Ancusa and Cynoglossum*, but the *Borago* (Boraginaceae) genus as well. The presence of **PDPGA** in different genera of Boraginaceae family would be interesting from the chemotaxonomic point of view. This unusual caffeic acid-derived polymer could be a chemotaxonomic marker among Boraginaceae plants. The biosynthetic pathway responsible for this compound might also be unique for different genera of Boraginaceae plants. Further detection of **PDPGA** amongst other members of the Boraginaceae family is interesting due to the importance of the chemotaxonomic significance, the potential biomedical applications of the Boraginaceae plants and the chemical importance

of **PDPGA**. Thus, the results of this study support the previous research that **PDPGA** can be used as a chemotaxonomic marker. The presence of poly[3-(3,4-dihydroxyphenyl)glyceric acid] in multiple Boraginaceae species expands the resources of raw materials for this biologically active polymer.

Materials and Methods

Apparatus: The UV spectra were 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 (Jasco, FT/IR-4600, Tokyo, Japan). All NMR spectra of 1% solutions in D_2O at 80°C and with acetone-d_6 were recorded in a Varian NMR System 500 MHz (Palo Alto, CA, USA) or Bruker Avance III 400 MHz (Uster, Switzerland). All NMR spectra were processed with the MestReNOVA software (version 14.2.1, Mestrelab Research, S. L., Santiago de Compostela, Spain). The ultrafiltration fractionation procedure was carried out in a stirred ultrafiltration cell (model 8200, Millipore Corporation, Billerica, MA, USA), fitted with a Biomax-500 ultrafiltration disc (500 000 NMWL).

Plant material: *B. officinalis* was cultivated near Tbilisi, Georgia. Fresh stems of *B. officinalis* were collected in June of 2019. A voucher specimen (TBPH №19568) was deposited at the Tbilisi State Medical University I. Kutateladze Institute of Pharmacochemistry.

Extraction and isolation: 33.5 g of air-dried and ground stems of *B. officinalis* were preliminary pretreated in a Soxhlet apparatus with chloroform, methanol and acetone, sequentially. Hot water extraction of preliminary pretreated 24.4 g of stems of *B. officinalis* followed by dialysis [1-5] afforded 6 g of crude polysaccharides of *B. officinalis* stems **(CP-BS)**. Yield of **CP-BS** was 17.9 %, based on air-dried biomass (6/33.5g). Further fractionation procedure of 0.794 g of **CP-BS** by ultrafiltration on a membrane filter with a cut off value of 500 kDa [1-5] afforded 0.087 g of high-molecular (>500 kDa) preparation of *B. officinalis* stems **HMP-BS**. Yield of **HMP-BS** based on crude polysaccharides (0.087/0.794g) was 11 %. Ultrafiltration of 6 g **CP-BS** afforded 0.66 g **HMP-BS.** Yield of **HMP-BS** based on air-dried biomass (0.66/33.5 g) was 2 %.

Carbohydrate analysis: The fructose content in **CP-BS** and **HMP-BS** was determined spectrophotometrically (11.6 and 2%, respectively) as described in previous papers [2-5]. Galacturonic acid in **CP-BS** and **HMP-BS** was also measured spectrophotometrically (16 and 5.4%, respectively) [2,3,5].

UV spectrum of **HMP-BS** (H_2O, λ_{max}, nm): 214, 236 (shoulder), 282 (shoulder), 286.

IR spectrum of **HMP-BS** (KBr, υ, cm^{-1}): 3553.7, 3477.0, 3414.8 (st OH); 2923 (CH); 1740 (–$COOCH_3$); 1636.8 (–COOH); 1618.5, 1508.5, 1440 (aromatic C=C); 1422.7, 1013 (phenols); 1266.5, 1147, 1099 (R-O-R'); 894 (C-H in the aromatic ring with one isolated hydrogen atom); 821.5 (C-H in the aromatic ring with two neighboring hydrogen atoms).

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ფარმაკოქიმია

ბიოპოლიმერები ნახშირწყლების საფუძველზე: *Borago officinalis*-ის ბიოლოგიურად აქტიური პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავა]

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უი, იწ, ^{1}H და ^{13}C ბმრ, gCOSY და 2D ჰეტერობირთვული ^{1}H/^{13}C gHSQCED ექსპერიმენტების მონაცემების საფუძველზე დადგენილ იქნა, რომ *symphytum asperum*, *S. caucasicum, S. officinale, S. grandiflorum, Anchusa italica* და *cynoglossum officinale*-ის (Boraginaceae) მაღალმოლეკულური ფრაქციების მსგავსად, *Borago officinalis*-ის ღეროების წყალში ხსნადი მაღალმოლეკულური პრეპარატის (მმპ-BO) ძირითადი კომპონენტია ბიოლოგიურად აქტიური კოფეინის მჟავას წარმოებულის პოლიმერი, კერძოდ, პოლი[ოქსი-1-კარბოქსი-2-[3,4-დიჰიდროქსიფენილ)ეთილენი], ანუ პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავა] (პდფგმ). მპდფგმ-ის დეტექტირება Boraginaceae-ს ოჯახის სხვადასხვა გვარში საინტერესოა ქემოტაქსონომიური თვალსაზრისით. კოფეინის მჟავას წარმოებულის უჩვეულო პოლიმერი შეიძლება იყოს Boraginaceae-ს მცენარეების ქემოტაქსონომიური მარკერი. ამგვარად, პდფგმ საინტერესოა ქიმიური და ქემოტაქსონომიური თვალსაზრისით, ხოლო Boraginaceae-ს მცენარეები მათი პოტენციური ბიოსამედიცინო გამოყენების მნიშვნელობის გამო. პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავას] არსებობა Boraginaceae-ს მრავალ სახეობაში აფართოებს ამ ბიოლოგიურად აქტიური პოლიმერის ნედლეულის რესურსების სიას.

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