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Biologically Active Caffeic Acid-Derived Biopolymer

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Abstract**—**The high-molecular water-soluble preparations from several species of two genera (*Symphytum* and *Anchusa*) of Boraginaceae family *Symphytum asperum*, *S. caucasicum*, *S.officinale* and *Anchusa italica* were isolated. According to IR, ^{13}C and ^{1}H NMR, APT, 1D NOE, 2D heteronuclear $^{1}H/^{13}C$ HSQC and 2D DOSY experiments, the main chemical constituent of these preparations was found to be caffeic acid-derived polyether, namely poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA) or poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene]. Most carboxylic groups of this caffeic acid-derived polymer of *A. italica* are methylated.

Keywords**—***Anchusa,* poly[3-(3,4-dihydroxyphenyl)glyceric acid], poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene], *Symphytum.*

I. Introduction

WITHIN the field of pharmacologically active biopolymers the area of stable polyethers seems rather new and attractive. In the last decade water-soluble high-molecular fractions from several species of two genera (*Symphytum* and *Anchusa*) of Boraginaceae family *Symphytum asperum (HM-SA)*, *S. caucasicum (HM-SC)*, *S. officinale (HM-SO)* and *A. italica (HM-AI)* roots were isolated [1]-[3]. The presented special communication summarizes data concerning novel caffeic acid-derived polyether – the main constituent of above mentioned preparations.

II. Materials and Methods

A. Materials

Roots of *S. asperum*, *S. caucasicum*, *S. officinale* and *A. italica* were collected in Ajara region (*S. asperum*) and Tbilisi suburbs (*S. caucasicum*, *S. officinale*, and *A. italica)* in May-June. Voucher specimens are stored at the Tbilisi State Medical University Institute of Pharmacochemistry Herbarium.

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B. General Experimental Procedures

Crude polysaccharides from the roots of *S. asperum*, *S. caucasicum*, *S. officinale* and *A. italica* were obtained as described in [1].

The high-molecular caffeic acid-derived preparations from crude polysaccharides were isolated by ultrafiltration on membrane filter with cut-off value of 1000 kDa.

UV spectra were recorded on a Hitachi 150-20 spectrophotometer; IR spectra were registered on a Jasco FT/IR-410 spectrophotometer; NMR were taken on a Bruker DRX-500 spectrometer for 1% solutions of the polymers in D_2O at 80ºC using acetone (δ_H 2.225 ppm, δ_C 31.45 ppm) as the internal standard. The 2D HSQC spectra were obtained using the Bruker standard software. Pre-irradiation time for the 1D NOE experiment was 1 s, the signal of pre-irradiated proton in the difference spectrum was taken as 100%. For the 2D DOSY experiments, a Bruker pulse sequence that incorporated stimulated echo and longitudinal eddy currents and bipolar gradients for diffusion was used for both optimization of diffusion time and gradient pulse duration (1D) and then diffusion measurement (2D).

CD spectra were performed on a Jasco J-715 instrument (Jasco Co., Tokyo, Japan) equipped with peltier temperature control system. For all measurements, 1 mm path length quartz cells, 1 nm bandwidth, 0.2 nm resolution, 1 s response and a scan speed of 50 nm/min for each spectrum were used.

III. Results and Discussion

The ultrafiltration of crude polysaccharides from *S. asperum*, *S. caucasicum*, *S. officinale* and *A. italica* allowed to remove most polysaccharides and to obtain biologically active water-soluble high-molecular preparations with molecular masses exceeding 1 MDa.

The UV spectra of *HM-SA, HM-SC, HM-SO* and *HM-AI* were identical to each other. They exhibited the same absorption maxima indicative of the phenolic nature of the preparations. IR spectra of *HM-SA, HM-SC, HM-SO* and *HM-AI* fractions were also identical and contained absorption bands typical of phenolcarboxylic acids [1]-[3].

The ^{13}C NMR spectra of *HM-SA*, *HM-SC*, *and HM-SO* were completely identical [1], [2]. Interestingly, the signals of the residual carbohydrate components are practically unobservable in the spectra of these preparations, probably due to their variegated monosaccharide composition; only nine distinct signals corresponding to the carbon atoms of the

substituted phenylpropionic acid fragment were observed (Fig. 1). It follows from the spectra obtained using the APT technique [1] (Fig. 2) that five signals should be assigned to CH groups and four signals to the nonprotonated carbon atoms. The two signals with chemical shifts of 78.2 and 80.4 ppm obviously belong to oxygen-bound protonated aliphatic carbon atoms. Six signals were assigned to aromatic carbon atoms (protonated atoms at 117.4, 118.6, and 122.3 ppm and nonprotonated atoms at 131.5, 143.8, and 144.6 ppm). The broadened signal at 175.4 ppm was assigned to the carboxyl group in the compound. The ^{1}H NMR spectra of *HM-SA*, *HM-SC*, *and HM-SO* were also practically identical (Fig. 3) [1], [2]. They contain four signals at 4.88, 5.33, 7.13, and 7.24 ppm, one of them (7.13 ppm) with doubled intensity. Unfortunately, these signals are broadened, and, therefore, the coupling constants cannot be determined. The 2D heteronuclear ^{1}H/^{13}C HSQC spectrum [1], [2] (Fig. 4) exhibits the following correlations between protons and carbon atoms: 4.88/80.4, 5.33/78.2, 7.13/118.6, 7.13/122.3, and 7.24/117.4 (ppm/ppm). The good resolution and the narrow shape of the ^{13}C-NMR signals indicate that the compounds under study are regular polymers. The polyoxyethylene chain is the backbone of the polymer molecule according to the spectral data. Dihydroxyphenyl and carboxyl groups are regular substituents at two carbon atoms in the chain (Fig. 5). The hydroxyl groups in positions 3 and 4 of the phenyl ring were unambiguously established by a 1D NOE experiment performed in the difference mode. The pre-irradiation of the proton at position 1 (5.33 ppm) caused a NOE in the two aromatic protons with the chemical shifts of 7.13 and 7.24 ppm [1]. Hence, these protons occupy positions 2 and 6 in phenyl ring. Therefore, hydroxyl groups cannot occupy *o*-positions. Different values of NOE for these protons, different chemical shifts, and different chemical shifts of the resonances of the corresponding carbon atoms in the ^{13}C NMR spectrum exclude the feasibility of a symmetric bis-*m*-substitution pattern in the aromatic ring with two hydroxyl groups. The total assignment of the complete set of resonances characteristic for poly[3-(3,4-dihydroxyphenyl)glyceric acid] in ^{13}C NMR and ^{1}H NMR spectra and the correlations between protons and carbon atoms that were established using 2D heteronuclear ^{1}H/^{13}C HSQC are listed in Table I (see also Fig. 5).

Thus, the main component of *HM-SA*, *HM-SC* and *HM-SO* is regular polymer poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] or poly[3-(3,4-dihydroxyphenyl)glyceric acid] **(***PDPGA***),** that is *PDPGA-SA*, *PDPGA-SC*, *PDPGA-SO***.** The repeating unit (Fig. 5) of this polyether contains two asymmetric carbon atoms C1 and C2. Comparative study of chirality of these atoms of poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] from *S.asperum, S.caucasicum, S.officinal* and *Anchusa italica* by circular dichroism (CD) revealed that they have one and the same absolute configuration. It has been established that chiral atoms of polymer have either (*1R,2R*) or (*1S,2S*) configurations and consequently denomination of polymer is poly[oxy-(*1R*)-1-carboxy-(*2R*)-2-(3,4-dihydroxyphenyl)ethylene] or poly[oxy-(*1S*)-1-carboxy-(*2S*)-2-(3,4-dihydroxyphenyl)ethylene] [4].

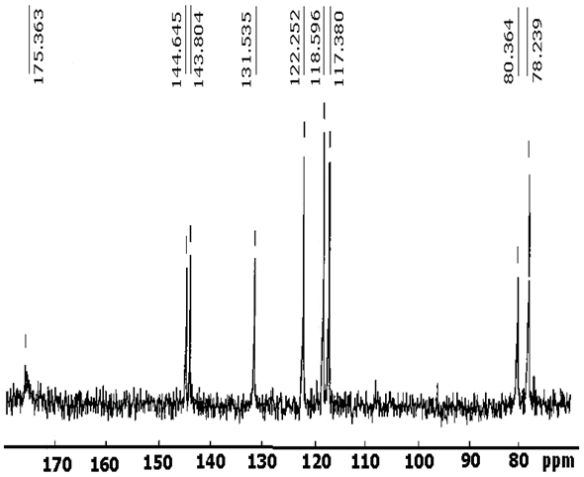

Fig. 1 The ^{13}C NMR spectrum of *HM-SA*, *HM-SC* and *HM-SO*

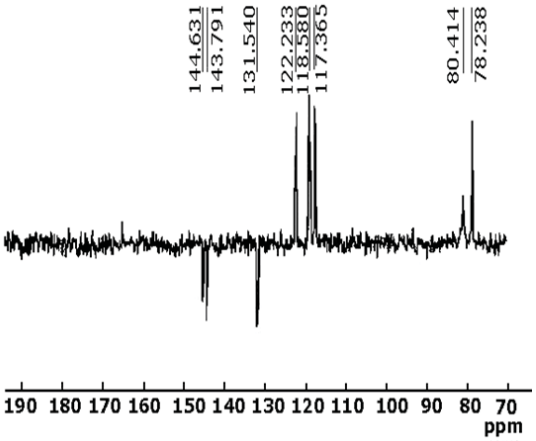

Fig. 2 The APT spectrum of *HM-SA*, *HM- HM-SC* and *HM-SO*

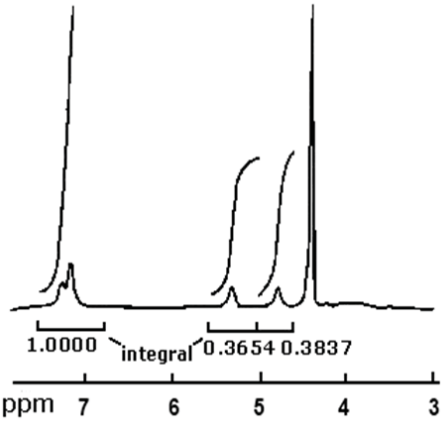

Fig. 3 The ^{1}H NMR spectrum of *HM-SA*, *HM- HM-SC* and *HM-SO*

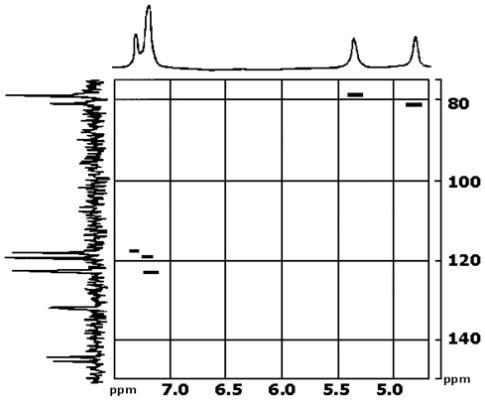

Fig. 4 The $^{1}H/^{13}C$ HSQC spectrum of *HM-SA*, *HM-SC* and *HM-SO*

HM-AI was characterized by using different NMR spectroscopy techniques. The ^{1}H NMR, ^{13}C NMR, and 2D heteronuclear $^{1}H/^{13}C$ HSQC spectra of *HM-AI* showed also a complete set of resonances characteristic for poly[3-(3,4-dihydroxyphenyl)glyceric acid] (Table I). However, in contrast to *Symphytum PDPGA,* non-sharp signals (172.8 and 175.6 ppm) were thought to be due to two carboxyl groups. A resonance in the ^{13}C NMR spectrum at 54.9 ppm, which correlated with the ^{1}H resonance at 3.85 ppm, suggested the presence of methoxy groups in carboxylic acid methyl esters. Thus, the signals at δ 175.6 and δ 172.8 were assigned to carboxylic acid groups and methyl ester carbonyl functions (shifted upfields), respectively [3].

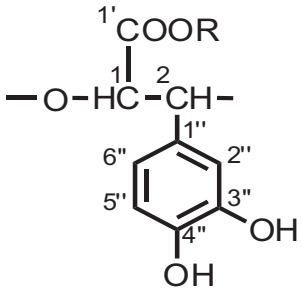

Fig. 5 The repeating unit of *PDPGA;* R=H, CH_3

TABLE I
SIGNALS ASSIGNMENT IN THE ^{13}C AND ^{1}H NMR SPECTRA OF PDPGA-SA, PDPGA-SC, PDPGA-SO

C Atom no.	^{13}C Chemical shift (δ, ppm)	^{1}H Chemical shift (δ, ppm).
1'	175.4	
1	78.2	5.33
2	80.4	4.88
1"	131.5	
2"	117.4	7.24
3"	144.6	
4"	143.8	
5"	118.6	7.13
6"	122.3	7.13

About 70 % of the present carboxyl groups were methyl esterified (MeO: ^{13}C, 54.86 ppm; ^{1}H, 3.85 ppm). The extent of methyl esterification was calculated by comparing the integral intensity of the methyl ester signal (3.85 ppm, 0.5 H) to that of the aliphatic proton signal at H1 (5.24 ppm, 0.7 H) in a ^{1}H experiment that included a WATERGATE water suppression routine. The presence of methoxy groups at C3" and/or C4" in the aromatic ring is excluded as there are no downfield shifts of the signals of C3" (δ 145.3) and/or C4" (δ 144.5), which would be expected from methylation of any of these hydroxyl groups. Moreover, the 2D DOSY experiment gave the similar diffusion coefficient for the methylated and non-methylated signals. Both sets of signals fell in the same horizontal. This would imply a similar (same order of magnitude) molecular weight for methylated and non-methylated polymers. This was further evidenced by graphic presentations of the intensity decay of the ^{1}H signals of aromatic H-2″ and H-1 at δ 7.16 and 5.24 (Fig. 5), and that of the methoxy group at δ 3.85. These three ^{1}H signals essentially showed the same curve shape, whereas the resonance due to residual water at δ 4.35 followed a different decay pattern (faster diffusion). Consequently, the NMR signals of both methylated and non-methylated carboxylic groups originate from the same poly[3-(3,4-dihydroxyphenyl)glyceric acid] polymer of *HM-AI* [3].

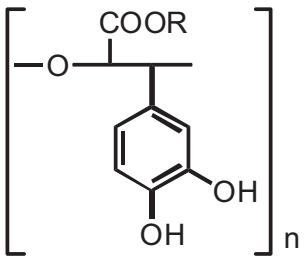

Fig. 6 *PDPGA* from *S. asperum, S. caucasicum*, *S. officinale* and *A. italica*; R=H, CH_3

PDPGA–SA, *PDPGA–SC* and *PDPGA–SO* exhibit immunomodulatory (anticomplementary), antioxidant, antiinflammatory activities [1], [2], [5], [6] and wound-healing property [7], [8].

The ability of cancer cells to metastasize, escape from the original tumors and spread in the body, makes cancer tenacious and deadly. *PDPGA–SA* showed anti-metastatic property *in vitro*. It completely abrogated the adhesion of murine B16 melanoma cells to tumor-activated hepatic sinusoidal endothelium (HSE), without any detectable effect on basal condition-cultured HSE. Consistent with these anti-adhesive effects, *PDPGA–SA* also prevented melanoma cell adherence to recombinant vascular endothelial growth factor (VEGF)-treated HSE [9]. We investigated the efficacy of *PDPGA–SC* and its synthetic monomer *syn*-2,3-dihydroxy-3-(3,4-dihydroxyphenyl) propionic acid *(MDPPA)* against androgen-dependent and -independent human prostate cancer (PCA) LNCaP and 22Rv1 cells. We found that both *PDPGA–SC* and *MDPPA* suppressed the growth and induced death in PCA cells, with comparatively lesser cytotoxicity towards non-neoplastic human prostate epithelial cells. Furthermore, we also found that both *PDPGA–SC* and *MDPPA* caused G1 arrest in PCA cells through modulating the expression of cell

cycle regulators, especially an increase in cyclin-dependent kinase inhibitors (CDKIs) (p21 and p27). In addition, *PDPGA–SC* and *MDPPA* induced apoptotic death by activating caspases, and also strongly decreased androgen receptor (AR) and prostate specific antigen (PSA) expression. Consistent with *in vitro* results, our *in vivo* study showed that *PDPGA–SC* feeding strongly inhibited 22Rv1 tumors growth by 76% and 88% at 2.5 and 5 mg/kg body weight doses, respectively, without any toxicity, together with a strong decrease in PSA level in plasma; and a decrease in proliferating cell nuclear antigen (PCNA), AR and PSA expression but increase in p21/p27 expression and apoptosis in tumor tissues from *PDPGA–SC*-fed mice. Thus, *PDPGA–SC* and its synthetic monomer exerted anti-cancer efficacy *in vitro* and *in vivo* against androgen-dependent and -independent PCA cells via targeting androgen receptor, cell cycle arrest and apoptosis without any toxicity, together with a strong decrease in PSA level in plasma. Overall, this study identifies *PDPGA–SC* as a potent agent against PCA without any toxicity, and supports its clinical application [10].

IV. Conclusion

Thus, one and the same biologically active caffeic acid-derived polymer, namely poly[3-(3,4-dihydroxyphenyl)glyceric acid] *(PDPGA)* or poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] (Fig. 6) is the main structural element of high-molecular water-soluble preparations isolated from the roots of different species of Boraginaceae family. This compound represents a class of natural polyethers with a residue of 3-(3,4-dihydroxyphenyl)glyceric acid as the repeating unit (Fig. 5). In contrast with the *Symphytum* polymer most of the carboxylic groups of this caffeic acid-derived polyether of *A. italica* are methylated. We have no information on the biosynthesis of such a polymer in plants, but, from the chemical viewpoint, this process can be conceived as the epoxidation of the double bond in caffeic acid followed by the polymerization of the resulting epoxide. Further study should clarify the physiological function of these polyethers in plants and demonstrate whether their biosynthesis is the unique property of the genera *Symphytum* and *Anchusa* or whether such compounds are also generated in other plants.

According to *in vitro* and *in vivo* experiments *PDPGA* could be considered as potential anti-inflammatory, wound healing and anti-cancer therapeutic agent.

References

[1] V. Barbakadze, E. Kemertelidze, I. Targamadze, K. Mulkijanyan, A. S. Shashkov, and A. I. Usov, "Poly(3-(3,4-dihydroxyphenyl)glyceric acid), a new biologically active polymer from *Symphytum asperum* Lepech. and *S.caucasicum* Bieb. (Boraginaceae)", *Molecules*, vol. 10, no. 9, pp. 1135-1144, Sept. 2005.

[2] V. Barbakadze, A. J. J. van den Berg, C. J. Beukelman, J. Kemmink, and H.C. Quarles van Ufford, "Poly(3-(3,4-dihydroxyphenyl)glyceric acid) from *Symphytum officinale* roots and its biological activity", *Chem. Nat. Compds*., vol. 45, no. 1, pp. 6-10, January-February 2009.

[3] V. Barbakadze, L. Gogilashvili, L. Amiranashvili, M. Merlani, K. Mulkijanyan, M. Churadze, A. Salgado, and B. Chankvetadze, "Poly(3-(3,4-dihydroxyphenyl)glyceric acid) from *Anchusa italica* roots", *Nat. Prod. Commun.,* vol. 5, no. 7, pp. 1091-1095, July 2010.

[4] V. Barbakadze, M. Merlani, L. Amiranashvili, L. Gogilashvili, and K. Mulkijanyan. "Study of poly(oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene) from *Symphytum asperum, S.caucasicum, S.officinale, Anchusa italica* by circular dichroism", *Bull. Georg. Natl. Acad. Sci.*, vol. 6, no. 1, pp. 143-146, January-April 2012.

[5] V. V. Barbakadze, E. P. Kemertelidze, K. G. Mulkijanyan, A. J .J. van den Berg, C. J. Beukelman, E. van den Worm, H. C. Quarles van Ufford, and A. I. Usov, "Antioxidant and anticomplementary activity of poly(3-(3,4-dihydroxyphenyl)glyceric acid) from *Symphytum asperum* and *S. caucasicum"*, *Pharm. Chem. J.,* vol. 41, no 1, pp. 14-16, January 2007.

[6] C. M. Barthomeuf, E.Debiton, V.V.Barbakadze, and E.P.Kemertelidze. "Evaluation of the dietetic and therapeutic potential of a high molecular weight hydroxicinnamate-derived polymer from *Symphytum asperum* Lepech. Regarding its antioxidant, antilipoperoxidant, antiinflammatory, and cytotoxic properties", *J. Agric. Food Chem.*, vol. 49, no. 8, pp. 3942-3946, August 2001.

[7] V. Barbakadze, K. Mulkijanyan, L. Gogilashvili, L. Amiranashvili, M. Merlani, Zh. Novikova, and M. Sulakvelidze, "Allantoin- and pyrrolizidine alkaloids-free wound healing compositions from *Symphytum asperum*", *Bull. Georg. Natl. Acad. Sci.*, vol. 3, no. 1, pp. 159-164, January-April 2009.

[8] K. Mulkijanyan, V. Barbakadze, Zh. Novikova, M. Sulakvelidze, L. Gogilashvili, L. Amiranashvili, and M. Merlani, "Burn healing compositions from Caucasian species of comfrey (*Symphytum* L.)", *Bull. Georg. Natl. Acad. Sci.,.* vol. 3, no. 3, pp. 114-117, September-December 2009.

[9] V. Barbakadze, K. Mulkijanyan, M. Merlani, L. Gogilashvili, L. Amiranashvili, and F. Vidal-Vanaclocha, "Effects of poly(3-(3,4-dihydroxyphenyl)glyceric acid) on the inflammatory response of tumor-activated hepatic sinusoidal endothelium", *Bull. Georg. Natl. Acad. Sci.,* vol. 2, no 3, pp. 108-112, July-September 2008.

[10] S. Shrotriya, G. Deep, K. Ramasamy, K. Raina, V. Barbakadze, M. Merlani, L. Gogilashvili, L. Amiranashvili, K. Mulkijanyan, K.Papadopoulos, C. Agarwal, and R. Agarwal. "Poly(3-(3, 4-dihydroxyphenyl) glyceric) acid from comfrey exerts anti-cancer efficacy against human prostate cancer via targeting androgen receptor, cell cycle arrest and apoptosis", *Carcinogenesis*, vol. 33, no. 8, pp. 1572-1580, August 2012.