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Pharmacochemistry

Novel Biologically Active Dihydroxycinnamate-Derived Polyether from Different Species of Family Boraginaceae

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ABSTRACT. Plants belonging to Boraginaceae family such as *Symphytum asperum*, *S.caucasicum*, *S.officinale* and *Anchusa italica* have been widely used in folk medicine for centuries. Recent pharmacological studies of water-soluble high-molecular fractions from the roots of these species revealed pronounced anticomplementary, antioxidant, anti-inflammatory and wound healing activity. However, the active principle responsible for the observed effects of these fractions was not known. The present special communication summarizes the phytochemical data of the last decade on novel dihydroxycinnamate-derived polyether – the main constituent of the above-mentioned preparations in order to identify the active principles responsible for their biological activity. Structural analysis of constituents of high-molecular fractions was made, based upon IR, ^{13}C, ^{1}H NMR, 2D heteronuclear $^{1}H/^{13}C$ HSQC spectra, 1D NOE and 2D DOSY experiments. The main chemical constituent of high-molecular water-soluble preparations from *S.asperum, S.caucasicum, S.officinale* and *A.italica* was found to be a novel dihydroxycinnamate-derived polyether, namely poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] or poly[3-(3,4-dihydroxyphenyl)glyceric acid]. In contrast to *Symphytum* polymer, most of the carboxylic groups of polymer from *A. italica* are methylated. *© 2013 Bull. Georg. Natl. Acad. Sci.*

Key words: *NMR, Boraginaceae,* Symphytum, Anchusa, *dihydroxycinnamate-derived polyether, poly[3-(3,4-dihydroxyphenyl)glyceric acid], poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene].*

Within the field of pharmacologically active biopolymers the area of stable polyethers seems rather new and attractive. Preliminary phytochemical and pharmacological studies of several plants belonging to Boraginaceae family, which are widely used in folk medicine, showed that from extracts of some representatives of Boraginaceae exhibited pronounced anticomplementary, antioxidant, anti-inflammatory, wound healing and anti-ulcer activity [1-5]. However, the active principle responsible for the observed effects was not known.

In the last decade water-soluble high-molecular

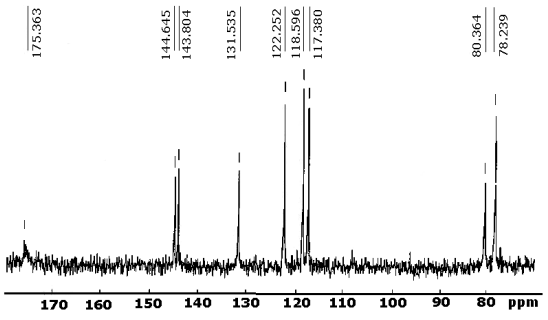

Fig. 1. The ^{13}C NMR spectrum of HM-SA, HM-SC and HM-SO.

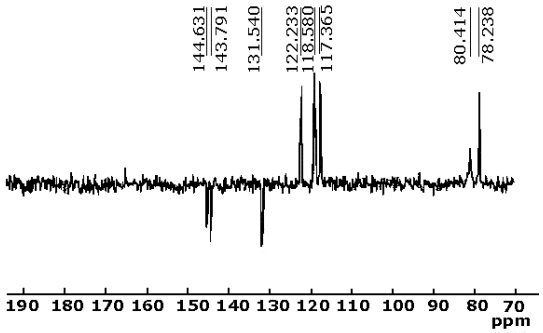

Fig. 2. The APT spectrum of HM-SA, HM-SC and HM-SO.

fractions from *S. asperum* (HM-SA), *S. caucasicum* (HM-SC), *S. officinale* (HM-SO) and *A. italica* (HM-AI) roots were isolated [6-10]. The present special communication summarizes data on novel dihydroxycinnamate-derived polyether – the main constituent of the above-mentioned preparations.

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 [6-10].

The ^{13}C NMR spectra of HM-SA, HM-SC, and HM-SO were also completely identical [6-9]. 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 [6-8] (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 [6-9] (Figure 3). 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 [6-9] (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. 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 [6-8]. Hence, these protons occupy positions 2 and 6 in the phenyl ring. Therefore, hydroxyl groups cannot occupy *o*-positions. Different values of NOE for these protons, different chemical

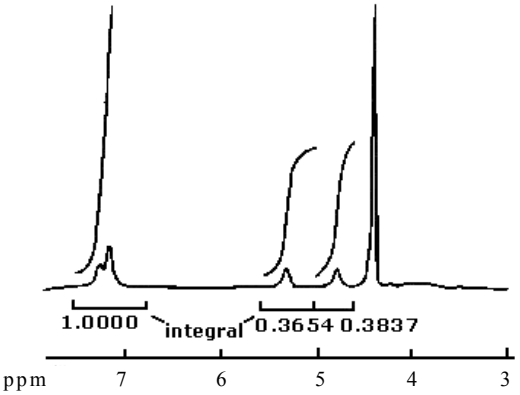

Fig. 3. The ^{1}H NMR spectrum of HM-SA, HM-SC and HM-SO.

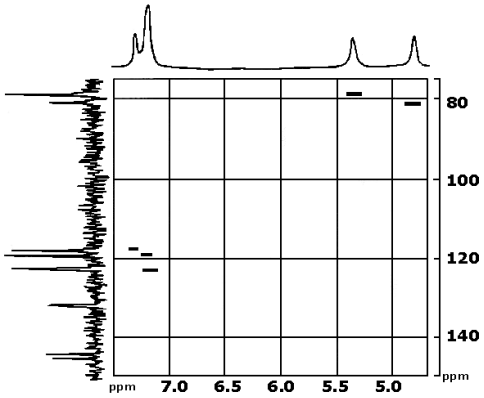

Fig. 4. The HSQC spectrum of **HM-SA, HM-SC** and **HM-SO**.

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 of 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 1 [6-9].

Thus, the main component of HM-SA, HM-SC and HM-SO is 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 [6-9].

The UV and IR spectra of HM-AI were similar to those of HM-SA, HM-SC and HM-SO.

The IR spectrum of HM-AI showed all the characteristic bands corresponding to the hydroxyl groups attached to the aromatic ring as well as the carboxyl and ether groups [10]. The IR spectrum contained bands at 1605 cm^{-1} for the COO^{-} and 1736 cm^{-1} for its ester form [11].

HM-AI was further characterized by using different NMR spectroscopy techniques [10]. The ^{1}H NMR, ^{13}C NMR, and 2D heteronuclear ^{1}H/^{13}C HSQC spec-

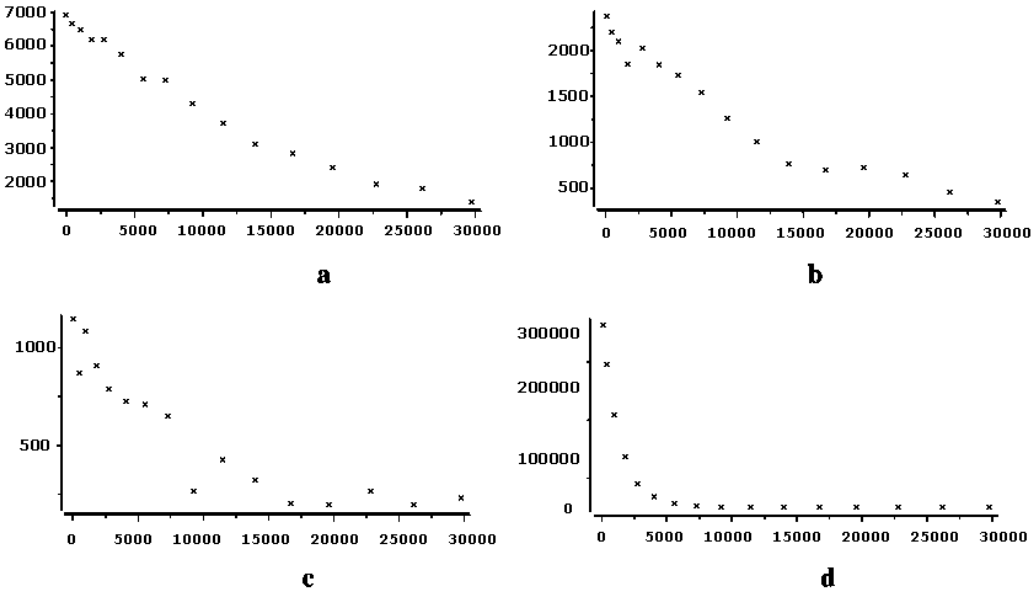

Fig. 5. Intensity decay of ^{1}H signals at 7.16 ppm (Ar H-2) (a), 5.24 ppm (H-1) (b), 3.85 ppm (OMe) (c), and 4.35 ppm (residual water) (d). X-axis, s/cm^{2}; Y-axis, relative intensity (dimensionless).

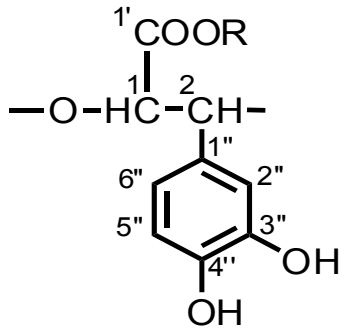

Fig. 6. The repeating unit of PDPGA; R=H, CH_3.

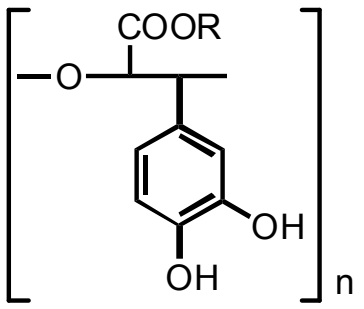

Fig. 7. Poly[3-(3,4-di-hydroxyphenyl)glyceric acid] (PDPGA) from *S. asperum, S. caucasicum*, *S. officinale* and *A. italica*; R=H, CH_3.

tra of HM-AI showed a complete set of resonances characteristic of poly[3-(3,4 dihydroxyphenyl)glyceric acid], which was earlier found in water-soluble high molecular fractions of *S. asperum, S. caucasicum* and *S. officinale* [6-9]. 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 175.56 and δ 172.84 were assigned to carboxylic acid groups and methyl ester carbonyl functions (shifted upfield), respectively [12-15]. 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 was excluded as there were no downfield shifts of the signals of C3" (S 145.3) and/or C4" (S 144.5), which would be expected from methylation of any of these hydroxyl groups [14,15]. Moreover, the 2D DOSY experiment gave a 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 representations of the intensity decay of the ^{1}H signals of aromatic H-2² and H-1 at 7.16 and 5.24 ppm (Figure 5a and b, respectively), and that of methoxy group at 3.85 ppm (Fig. 5c). These three ^{1}H signals essentially showed the same curve shape, whereas the resonance due to residual water at 4.35 ppm (Fig. 5d) followed a different decay pattern (faster diffusion).

Thus, the NMR signals of both methylated and non-methylated carboxylic groups originate from the same poly[3-(3,4-dihydroxyphenyl)glyceric acid] polymer.

The total assignment of the complete set of resonances characteristic of poly[3-(3,4-dihydroxyphenyl)glyceric acid] of HM-SA, HM-SC, HM-SO and HM-AI 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 spectrum are listed in Table 1 (see also Fig. 6).

It is necessary to emphasize that PDPGA–SA, PDPGA–SC and PDPGA–SO possessed anticomplementary, antioxidant and antiinflammatory activity [5,8,9,16,17] and wound-healing property [5,18,19].

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 the 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 condi-

Table 1. PDPGA signals assignment in the ^{13}C and ^{1}H NMR spectra of HM-SA, HM-SC, HM-SO and HM-AI (δ, ppm).

C atom no.	^{13}C chemical shift		^{1}H chemical shift	
	HM-AI	HM-SA, HM-SC, HM-SO	HM-AI	HM-SA, HM-SC, HM-SO
1'	175.6 (COOH) 172.8 ($COOCH_3$) 54.9 (OCH_3)	175.4 (COOH)	3.85 (OCH_3)	
1	78.8	78.2	5.24	5.33
2	80.9	80.4	4.71	4.88
1''	132.2	131.5		
2''	118.0	117.4	7.16	7.24
3''	145.3	144.6		
4''	144.5	143.8		
5''	119.2	118.6	7.06	7.13
6''	122.9	122.3	7.06	7.13

tion-cultured HSE. Consistent with these anti-adhesive effects, PDPGA–SA also prevented melanoma cell adherence to recombinant vascular endothelial growth factor (VEGF)-treated HSE [20].

PDPGA–SC and its synthetic monomer *syn*-2,3-dihydroxy-3-(3,4-dihydroxyphenyl)propionic acid exerted anti-cancer efficacy *in vitro* and *in vivo* against androgen-dependent and -independent human prostate cancer cells via targeting androgen receptor, cell cycle arrest and apoptosis without any toxicity, together with a strong decrease in prostate specific antigen (PSA) level in plasma [21].

Besides, antioxidant activity of PDPGA–AI was investigated against the relatively stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) in accordance with [22]. IC_{50} value of PDPGA–AI was 51.5μg/ml ± 1.11μg/ml (n=4).

Thus, one and the same novel biologically active dihydroxycinnamate-derived polyether, namely poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA) or poly[oxy-1-carboxy-2- (3,4dihydroxyphenyl) ethylene] (Fig. 7) 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 new class of natural polyethers with a residue of 3-(3,4-dihydroxyphenyl) glyceric acid as the repeating unit (Fig. 6).

The active principle responsible for the anticomplementary, antioxidant, anti-inflammatory and wound-healing activity of water-soluble high-molecular fractions from the roots of *S. asperum, S. caucasicum, S.officinale* and *A. italica* is a novel dihydroxycinnamate-derived polyether, namely poly[oxy-1-carboxy-2-(3,4dihydroxyphenyl) ethylene] or poly[3-(3,4-dihydroxyphenyl)glyceric acid].

There is 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 dihydroxycinnamic acid followed by the polymerization of the resulting epoxide. Moreover, the detection of poly[3-(3,4-dihydroxyphenyl)glyceric acid] in different genera of Boraginaceae family would be interesting from the chemotaxonomic point of view, as this unusual dihydroxycinnamate-derived polyether could be considered as a chemotaxonomic marker for Boraginaceae. Besides, its presence in different Boraginaceae genera expands the source of raw materials of this biologically active polymer.

ფარმაკოქიმია

Boraginaceae-ს ოჯახის ზოგიერთი სახეობის ახალი ბიოლოგიურად აქტიური დიჰიდროქსიდარიჩინატის წარმოებულის მარტივი პოლიეთერი

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Boraginaceae-ს ოჯახის მცენარეები *Symphytum asperum*, *S.caucasicum*, *S.officinale* და *Anchusa italica* საუკუნეების მანძილზე ფართოდ გამოიყენება ხალხურ მედიცინაში. ამ სახეობების მცენარეების ფესვების წყალში ხსნადი მაღალმოლეკულური ფრაქციების ფარმაკოლოგიურმა კვლევებმა აჩვენა, რომ მათ გააჩნიათ მკვეთრად გამოხატული ანტიკომპლემენტარული, ანტიოქსიდანტური, ანთების საწინააღმდეგო და ჭრილობის შემახორცებელი აქტივობები. მაგრამ ამ ფრაქციების მოქმედი საწყისი, რომელიც პასუხისმგებელია აღნიშნულ ეფექტებზე, ცნობილი არ იყო. მოცემული პუბლიკაცია არის ბოლო ათწლეულის ფიტოქიმიური მონაცემების შეჯამება, რომელიც ეძღვნება ზემოთ აღნიშნული პრეპარატების ძირითად შემადგენელ კომპონენტს – დიჰიდროქსიდარიჩინატის წარმოებულის მარტივ პოლიეთერს. ამ ფრაქციების ბიოლოგიურ აქტივობაზე პასუხისმგებელი მოქმედი საწყისის იდენტიფიცირების მიზნით ჩატარდა ამ მაღალმოლეკულური ფრაქციების შემადგენელი კომპონენტების სტრუქტურული ანალიზი ინფრაწითელი, ^{13}C, ^{1}H ბირთვულ-მაგნიტური რეზონანსის, 2D ჰეტერობირთვული $^{1}H/^{13}C$ HSQC სპექტრებისა და 1D NOE და 2D DOSY ექსპერიმენტების მონაცემების საფუძველზე. აღმოჩნდა, რომ *S.asperum*-ის, *S.caucasicum*-ის, *S.officinale*-ის და *A.italica*-ს წყალშიხსნადი მაღალმოლეკულური ფრაქციების ძირითადი ქიმიური კომპონენტია დიჰიდროქსიდარიჩინატის წარმოებულის მარტივი პოლიეთერი, კერძოდ, პოლი[ოქსი-1-კარბოქსი-2-(3,4-დიჰიდროქსიფენილ)ეთილენი] ანუ პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავა]. *Symphytum*-ის პოლიმერისაგან განსხვავებით *A.italica*-ს პოლიმერის კარბოქსილის ჯგუფების უმეტესი ნაწილი მეთილირებულია.

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