Chemistry of Natural Compounds, Vol. 41, No. 4, 2005

POLY[3-(3,4-DIHYDROXYPHENYL)GLYCERIC ACID] FROM STEMS OF *Symphytum asperum* AND *S. caucasicum*

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UDC 547.588.15:547.992

Two high-molecular-weight (>1000 kDa) water-soluble preparations were isolated from stems of Symphytum asperum *and* S. caucasicum. *Their basic component was established as poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene], analogous to that of high-molecular-weight (>1000 kDa) preparations from roots of these same plants, using IR and NMR spectral data.*

Keywords: caffeic acid, 3-(3,4-dihydroxyphenyl)glyceric acid, *Symphytum asperum*, *Symphytum caucasicum*, poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene]

Caucasian species of comfrey from the genus *Symphytum* L. have been used since antiquity to treat wounds and fractures. It was found that they possess analgesic, antimicrobial, and anti-inflammatory activities. They are also used for contusions, hematomas, ulcers, gastrointestinal infections, neuralgia, parodontosis, arthritis, and rheumatism [1].

Despite the popularity of these plants in folk medicine, literature data on their chemical composition and biological activity are rather scant.

Total polysaccharide preparations from roots of rough comfrey (*S. asperum* Lepech.) and Caucasian comfrey (*S. caucasicum* Bieb.) (Boraginaceae) exhibited anticomplementary activity [2, 3]. Both preparations were fractionated and monitored for activity in order to establish the active principle. Ultrafiltration on membrane filters (1000 kDa pores) followed by dissolution of high-molecular-weight components in veronal—salt buffer (pH 7.35) and gel-permeation chromatography removed most ballast polysaccharides and afforded water-soluble high-molecular-weight (>1000 kDa) fractions. Both fractions contained some residual carbohydrates (25.7 and 26.9%, respectively) and possessed high anticomplementary activities [2, 3]. One fraction from roots of *S. asperum* also exhibited antioxidant, antilipoperoxidant, and anti-inflammatory activities [4]. The UV spectra of both preparations contained absorption maxima at 252, 282 (sh), and 286 nm in veronal—salt buffer (pH 7.35) and at 213, 237, 282 (sh), and 286 nm in water. We demonstrated previously on the basis of IR and NMR spectral data that the principal component of both high-molecular-weight water-soluble preparations from roots of *S. asperum* and *S. caucasicum* was a regularly substituted polyoxyethylene, namely poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] [5, 6]. This previously unknown natural phenolic biopolymer was isolated for the first time, is a polymer derived from caffeic acid, and represents a new class of natural esters with 3-(3,4-dihydroxyphenyl)glyceric acid as the repeating unit.

The goal of the present investigation was to isolate water-soluble high-molecular-weight (>1000 kDa) preparations from the total polysaccharides from stems of rough comfrey (TRC) and Caucasian comfrey (TCC) and to compare the structures of the principal structural units using IR and NMR spectroscopies.

TRC and TCC were isolated from total polysaccharides of *S. asperum* and *S. caucasicum* stems using the previously reported method [2, 3].

Table 1 gives the amount of residual total sugars, fructose, uronic acid, and other monosaccharides in TRC and TCC.

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TABLE 1. Monosaccharide Composition of TRC and TCC Preparations

Preparation	Total sugars, %	Monosaccharide, %							
		Uronic acid	Fru	Rha	Ara	Xyl	Man	Glc	Gal
TRC	27.8	14.5	2.5	0.6	0.7	0.3	5.5	1.8	1.9
TCC	28.6	13.6	2.2	1.1	1.7	0.3	4.5	1.4	3.8

TABLE 2. Assignment of ^{13}C NMR and PMR Signals for TRC and TCC (δ, ppm)

C atom	δ_C	δ_H
1′	175.1	
1	78.1	5.20
2	80.4	4.62
1″	131.5	
2″	117.1	7.13
3″	144.6	
4″	143.8	
5″	118.3	7.00
6″	122.0	7.00

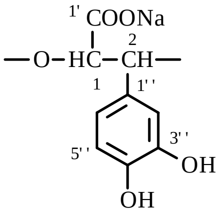

Fig. 1. Repeating unit of the polymer from *S. asperum* and *S. caucasicum*.

The TRC and TCC preparations contain an insignificant amount of fructose compared with the starting total polysaccharides in which fructose and uronic acid dominate [7].

The UV spectra of TRC and TCC were identical to each other and similar to analogous spectra of polyethers from roots of *S. asperum* and *S. caucasicum*. They exhibited the same absorption maxima in veronal—salt buffer (pH 7.35) and water, indicative of the phenolic nature of the preparations [2, 3].

IR spectra of the TRC and TCC fractions wee also identical and contained absorption bands typical of phenolcarboxylic acids. The bands were similar to those in IR spectra of the previously isolated polyethers from roots of rough comfrey and Caucasian comfrey [5, 6].

TRC and TCC were further characterized using ^{13}C NMR and PMR and two-dimensional (2D) heteronuclear ^{1}H/^{13}C HSQC.

The resulting spectra of TRC and TCC were practically identical and were also similar to NMR spectra of polyethers from roots of *S. asperum* and *S. caucasicum*.

Table 2 and Fig. 1 give the assignments of signals in the ^{13}C NMR and PMR spectra [5, 6].

It is noteworthy that signals from the carbohydrate components are practically unobserved in the spectra of these preparations because of the varied monosaccharide composition of TRC and TCC (Table 1).

The ^{13}C NMR spectra also exhibit nine distinct signals for C atoms of substituted phenylpropionic acid, namely 3-(3,4-dihydroxyphenyl)glyceric acid. Two signals belong to protonated aliphatic C atoms bound to oxygen; six, to three protonated and three unprotonated C atoms of the aromatic ring. A broadened signal at 175.1 ppm belongs to the carboxylic group.

The PMR spectrum contains four signals, one of which (7.00 ppm) is twice as strong as the others. Unfortunately, these signals are broadened. Therefore, it was impossible to determine the spin—spin coupling constants.

Table 2 lists the complete assignment of signals in the ^{13}C NMR and PMR spectra of TRC and TCC preparations and correlations between protons and C atoms found using 2D heteronuclear $^{1}H/^{13}C$ HSQC spectra.

The good resolution and narrow lines in the ^{13}C NMR spectra indicate that the substances are regular polymers. According to the spectral data, the polymer is based on poly(oxyethylene). Two C atoms of this chain are regularly substituted by 3,4-dihydroxyphenyl and carboxyl groups, respectively.

Our assignment of resonances in the ^{13}C NMR spectrum is consistent with existing spectral data in the literature for certain low-molecular-weight natural compounds that contain 3-(3,4-dihydroxyphenyl)lactic [10] and 3-(3,4-dihydroxyphenyl)glyceric acids [11] and caffeic acid derivatives [12].

Thus, ^{13}C NMR and PMR spectra in addition to 2D heteronuclear $^{1}H/^{13}C$ HSQC spectra of TRC and TCC were identical to those of high-molecular-weight fractions from roots of *S. asperum* and *S. caucasicum*, which consisted mainly of poly[hydroxy-1-carboxy-2-(3,4-dhydroxyphenyl)ethylene]. Therefore, we concluded that the main component of TRC and TCC is the same polyester. Its repeating unit is 3-(3,4-dihydroxyphenyl)glyceric acid and contains two asymmetric C atoms. However, we do not yet have data about the chirality of these centers. Establishing the absolute configuration of these C atoms and evaluating the structural significance of the residual carbohydrates are topics for further research.

The observation of a new unusual water-soluble high-molecular-weight polyether, a caffeic acid derivative, is very important from economic and ecological viewpoints because the isolation of biologically active compounds from stems of perennially renewable vegetative plant parts, firstly, is simpler and cheaper and, secondly, enables the plant population to be maintained.

EXPERIMENTAL

Isolation of Total Polysaccharides. Total polysaccharide preparations of *S. asperum* and *S. caucasicum* stems were obtained (6.5 and 6.8%, respectively, of the air-dried stem mass) as described previously [7, 8].

Isolation of TRC and TCC Preparations. TRC and TCC preparations (as sodium salts) from total polysaccharide preparations of *S. asperum* and *S. caucasicum* stems were isolated as before [2, 3]. Ultrafiltration on membrane filters (1000 kDa pores) followed by fractional dissolution in veronal—salt buffer (pH 7.35) and gel filtration over Sepharose 2B afforded total polysaccharides (25.7 and 22.8%, respectively), yield of TRC and TCC, 1.67 and 1.55%, respectively, of the dry stem mass.

Determination of Carbohydrate Composition of TRC and TCC. Fructose [13] and uronic acid [14] were determined colorimetrically.

Other monosaccharides were analyzed by hydrolyzing samples (5 mg each) in CF_3COOH (2 M) for 1 h at 121°C. Sugars were converted to acetates and determined by GC using myo-inosite as an internal standard [7, 8]. GC was performed in a gas chromatograph (Hewlett—Packard 5890) with a flame-ionization detector, capillary column (Ultra-1), and integrator (HP 3393A). The carrier gas was N_2. Chromatography conditions: 150°C (1 min) → 290°C (5°C/min).

UV, IR, and NMR Spectra of TRC and TCC. UV spectra were recorded on a Hitachi 150-20 spectrophotometer; IR spectra, in KBr disks on a Jasco FT/IR-410 spectrophotometer; NMR spectra, as solutions (1%) of polymeric caffeic-acid derivatives on a Varian Unity Inova 500 spectrometer (working frequency 500.13 mHz for ^{1}H and 125.76 MHz for ^{13}C) in D_2O at 70-80°C using acetone as an internal standard (δ_H 2.225 pm, δ_C 31.45 ppm from Me_4Si). 2D heteronuclear HSQC spectra were obtained using standard Varian programs.

UV spectrum (veronal—salt buffer, pH 7.35, λ_{max}, nm): 252, 282 (sh), 286; (H_2O, λ_{max}, nm): 213, 237, 282 (sh), 286.

IR spectrum (KBr, ν, cm^{-1}): 3408 (OH); 2927 (CH); 1615 (ionized carboxyl); 1509, 1443 (aromatic C=C); 1411, 1219 (phenol); 1269, 1098, 1075, 1017 (R–O–R′); 894 (C–H in aromatic ring with one isolated H atom); 822 (C–H in aromatic ring with neighboring H atoms).

Table 2 gives the PMR spectrum (500.13 MHz, D_2O, δ, ppm) and ^{13}C NMR spectrum (125.76 MHz, D_2O, δ, ppm).

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