*Chemistry of Natural Compounds, Vol. 46, No. 2, 2010*

# BRIEF COMMUNICATIONS

## CHEMICAL COMPOSITION OF *Callisia fragrans* JUICE. II. CARBOHYDRATES

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UDC 582.565.2:547.917

In continuation of research on the chemical composition of juice from *Callisia fragrans* Wood. (Commelinaceae) [1], we studied its carbohydrate components.

Polysaccharides were isolated by concentrating juice (21.5 L from 27 kg of fresh raw material) to a volume of 1.5 L; treating with hexane, $CHCl_3$, and EtOAc; and precipitating with EtOH (95%, 1:4). The resulting solid was centrifuged and dried by exchanging solvents to afford 45.11 g of polysaccharide complex CFP (9.42% of dry juice mass, 0.17% of fresh raw material mass). CFP was characterized by a high content of ash elements (54.3% including Al 0.11, Ba 0.20, Ca 24.47, Cr $2.64\cdot10^{-3}$, Cu $3.04\cdot10^{-3}$, Fe 0.22, K 9.14, Mg 9.22, Mn 0.10, Mo $2.07\cdot10^{-4}$, Na 2.77, Ni $1.06\cdot10^{-4}$, P 7.04, Pb $8.71\cdot10^{-4}$, Si 2.70, Sn $2.02\cdot10^{-4}$, Ti 0.01, V $2.62\cdot10^{-3}$, and Zn 0.02% of total ash mass) and protein (12.4%). Carbohydrate components made up 28.2% of the CFP mass including hexosamines (16.5%) and uronic acids (4.3%). Demineralization and removal of proteins from CFP produced fraction CFP′ in 25.3% yield.

**CFP′.** Neutral monosaccharides 28.3%, uronic acids 17.6%, hexosamines 52.9%. IR spectrum (ν, $cm^{-1}$): 600, 667, 716, 773 ($\beta$-bond), 795, 847 ($\alpha$-bond), 907, 947, 1027, 1083 (pyranose), 1267, 1300, 1417, 1574 (amide II), 1635 (COO), 1652 (amide I), 1744 (ester C=O), 2213, 2933 (C–H), 3210 (N–H), 3382 (N–H), 3452 (OH). Gel chromatography (MW, kDa): 61.2, 44.0, 39–32, 27.6. Content of acetyl groups: 3.06% (IR spectroscopy), 2.82% (hydroxylamine method).

According to gel chromatography, CFP′ was a heterogeneous polysaccharide fraction, the IR spectrum of which showed absorption bands for $\alpha$- and $\beta$-bonds, pyranose rings, and amide I and II in addition to bands specific for hexosamine polysaccharides (716, 795, 1300, 1417 $cm^{-1}$) [2]. After hydrolysis of CFP′ in $H_2SO_4$ (2 M, 100°C, 10 h), galacturonic acid, galactose, arabinose, and glucose (4.6:3.5:2.9:1) in addition to xylose, rhamnose, and glucosamine were detected. Using conc. HCl as the hydrolyzing agent (40°C, 1.5 h) produced glucosamine and galactosamine in a 7.8:1 ratio (Table 1). CFP′ was fractionated using precipitation by EtOH, which produced two subfractions CFP′-1 ([EtOH] 41%) and CFP′-2 ([EtOH] 63%) in 69.7 and 18.4% yields of CFP′ mass.

Fraction CFP′-1 was characterized by a high content of glucosamine (51.4%) and neutral carbohydrates (32.7%; Gal–Ara–Glc–Rha–Xyl 7:6:1.7:1.5:1). Glucuronic acid (33.1%) dominated in CFP′-2. Gel chromatography showed that CFP′-1 contained a minor component CFP′-1-1 (MW 61.2 kDa) and a dominant one CFP′-1-2 (MW 44 kDa), which were separated by preparative gel chromatography.

CFP′-1-2 contained neutral monosaccharides (30.1%, Gal–Ara–Glc 4.2:3.7:1) and hexosamines (67.8%, GlcN–GalN 8.4:1). IR spectrum (ν, $cm^{-1}$): 604, 671, 717, 775, 793, 845, 910, 953, 1025, 1091, 1259, 1301, 1375, 1418, 1560, 1650, 2210, 2917, 3207, 3401. Treatment of CFP′-1-2 with $H_2SO_4$ (3M, 100°C, 8 h) and subsequent precipitation with acetone produced homogeneous component CFP′-1-2d (62.4% yield of CFP′-1-2 mass, MW 25 kDa), which consisted of only glucosamine. The IR spectrum of CFP′-1-2d was similar to that of chitin (ν, $cm^{-1}$): 467, 578, 720, 800, 901, 1080, 1152, 1258, 1300, 1377, 1457, 1538, 1632, 1660, 2361, 2849, 2918, 3198, 3429; chitin: 467, 582, 718, 799, 900, 1093, 1154, 1258, 1300, 1376, 1457, 1535, 1637, 1652, 2360, 2847, 2922, 3202, 3419).

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0009-3130/10/4602-0273 ©2010 Springer Science+Business Media, Inc. 

TABLE 1. Monosaccharide Composition of *Callisia fragrans* Polysaccharide Fractions

| Fraction | Monosaccharide composition, mol% | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ara | Gal | Glc | Rha | Xyl | GalUA | GalN | GlcN |
| CFP′ | 11.1 | 13.4 | 3.8 | Tr. | Tr. | 17.6 | 6.1 | 46.8 |
| CFP′-1 | 11.4 | 13.3 | 3.3 | 2.8 | 1.9 | 3.7 | 7.4 | 51.4 |
| CFP′-1-1 | 25.2 | 29.7 | 9.7 | 3.3 | 2.3 | 29.7 | – | – |
| CFP′-1-2 | 12.5 | 14.2 | 3.4 | – | – | – | 7.2 | 60.6 |
| CFP′-1-2d | – | – | – | – | – | – | – | 99.9 |
| CFP′-2 | 20.3 | 22.2 | 4.4 | 1.7 | 0.8 | 33.1 | 1.3 | 12.4 |

Tr.: traces.

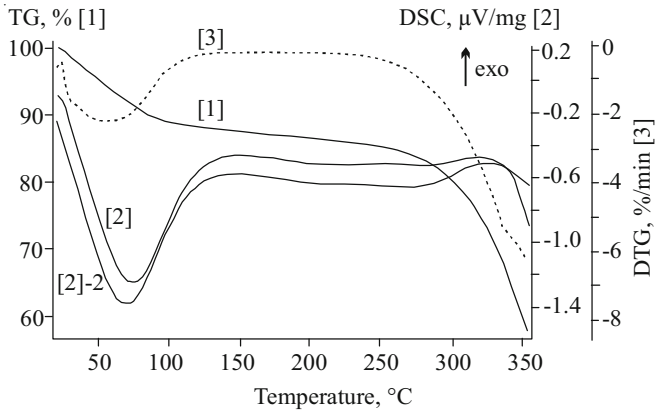


Fig. 1

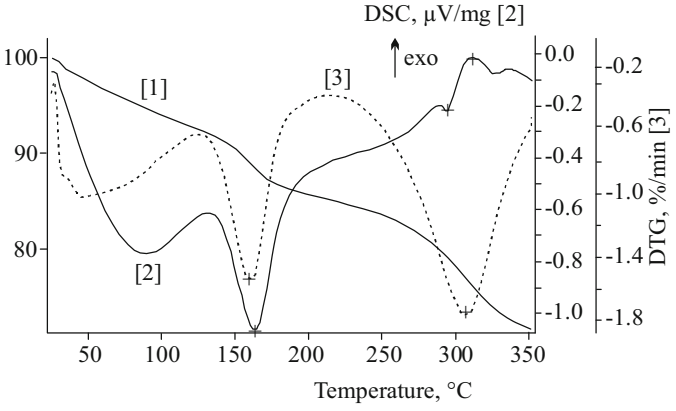


Fig. 2

Fig. 1. TG([1])-, DSC([2])-, and DTG([3])-thermograms for CFP′-1-2d ([2]-2, DSC-thermogram for chitin).
Fig. 2. TG([1])-, DSC([2])-, and DTG([3])-thermograms for CFP′-1-2.

Thermal analytical methods (TG, DTG, DSC) established that the DSC curve for CFP′-1-2d showed an exothermic maximum at 300–330°C (321.2°C) of height 0.21 V/g and an endothermic maximum at 60–90°C (74.3°C) that were typical of chitin (Fig. 1) [3]. Both maxima were found on the DSC curve of starting polymer CFP′-1-2 (312.3 and 88.7°C), which also showed a distinct endothermic peak at 163.3°C that was typical of galactans and uronide-containing polymers [4]. The DTG curve of CFP′-1-2 (Fig. 2) contained three portions of increased rate of mass loss at 48.5°C (0.93%/min, I), 160.1°C (1.53%/min, II), and 307.3°C (1.76%/min, III). These were due to loss of moisture (I) and destruction of polymer chains containing neutral carbohydrates (II) and thermally more stable hexosamines (III). The last condition was confirmed by the nature of mass loss for CFP′-1-2d, for which only two regions at 62.3°C (2.11%/min) and 331.2°C (5.60%/min) were found in this temperature range.

The $^{13}C$ NMR spectrum of CFP′-1-2d exhibited chemical shifts at 103.9 (C-1), 56.4 (C-2), 74.2 (C-3), 83.3 (C-4), 77.4 (C-5), and 60.7 (C-6) that characterized the polymer as linear $\beta$-(1→4)-glucosaminan [5].

The investigations found that polysaccharide juice of *C. fragrans* contained a complex set of macromolecular neutral and acidic components. The dominant polymer of CFP′-1-2 was a polysaccharide consisting of neutral monosaccharides and hexosamines, the main chain of which contained $\beta$-(1→4)-bonded glucosamine.

Free carbohydrates of *C. fragrans* juice consisted of glucose, galactose, glucosamine, and traces of galactosamine. The content of neutral carbohydrates in the juice was 0.56%; hexosamines, 0.35% (25.1 and 15.7% of the dry juice mass).

It was shown earlier that glucosamine and chitin and chitosan derivatives had a noticeable effect on immunity and nonspecific protective factors in vivo [6, 7]. Pharmacological studies found a pronounced adaptogenic activiy for *C. fragrans* juice with increased nonspecific resistance in vivo to the action of stress factors of various nature [8]. This was probably a result of the presence of free and bound hexosamines.

The aerial part of *C. fragrans* was supplied by the experimental plot at Vavilov SSAU (Saratov, Russia). Spectrophotometric studies were carried out on a UV-Vis-mini spectrophotometer (Shimadzu). Elemental composition was determined after ashing on a DFS-8 spectrograph. IR spectra were recorded in KBr disks (1:100) on a Spectrum 100 IR-Fourier spectrometer (Perkin–Elmer). Thermal analysis of polysaccharides was performed on an STA 449C derivatograph (Netzsch) under Ar in Pt–Rh crucibles for scan range 25–350°C and heating rate 10°C/min. $^{13}C$ NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz for DMSO-$d_6$ solutions (1%). Carbohydrate content was determined by the anthrone method [9]; uronic acids, by reaction with 3,5-dimethylphenol [10]; hexosamines, according to Elson–Morgan [11]; acetyl groups, by hydroxylamine [12] and IR spectroscopy methods [13]; quantitative monosaccharide composition, by HPTLC [14] and GC/MS (5973N GC/MS with Agilent Technoologies 6890N mass-selective detector with a diffusion pump, PH-Innowax capillary column, 30 m/250 μm/0.50 μm; 150–250°C temperature gradient, 2°C/min heating rate, He carrier gas, flow rate 1 L/min). Analytical gel chromatography was carried out over Sephadex G-100 (Pharmacia, 1.5 × 90 cm column); preparative, over Acrylex P-60 (Reanal, 4 × 80 cm column) with acetate buffer eluent (0.2 M NaOAc–2% AcOH, 1:1) and detection of yields by phenol–$H_2SO_4$ [15] and Elson–Morgan [11] methods.

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