A FRUCTOFURANAN FROM THE ROOTS OF *Rudbeckia fulgida,* VAR. *sullivantii* (BOYNTON ET BEADLE)

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Received April 29, 1997
Accepted July 20, 1997

From the roots of *Rudbeckia fulgida*, var. *sullivantii* (BOYNTON et BEADLE), a homogeneous polysaccharide has been isolated by water extraction and subsequent ion-exchange chromatography and gel filtration of the crude mixture of polysaccharides. Its molar composition determined by NMR and HPLC analyses was found to be D-fructose and D-glucose in the ratio 26 : 1. The results of methylation analysis and ^{1}H and ^{13}C NMR spectral measurements showed that the polysaccharide is a (2 → 1)-β-D-fructofuranan of the inulin type.
Key words: Natural products isolation; Plant polysaccharide; Fructofuranan; *Rudbeckia fulgida*.

In our continuing program on isolation of new, potentially active polysaccharides from various medicinal plants, we have recently found that the water-extractable polysaccharide complex from the aerial parts of *Rudbeckia fulgida*, var. *sullivantii* (BOYNTON et BEADLE) possessed high antitussive activity[1]. Other authors[2] reported on significant immunostimulating activity of aqueous-ethanol extracts from the roots of some *Rudbeckia* species. In view of these findings and complex utilization of the medicinal plant, it seemed reasonable to investigate also the roots of the title herb for the polysaccharide content. The present communication provides results on isolation and structure identification of the main neutral component of the water-extractable polysaccharide mixture, a fructofuranan of the inulin type. Inulin, present in the roots and tubers of many plant families[3,4] as a reserve polysaccharide, has been used mostly in the food industry and in diagnostic tests for renal function[5]. However, it is conceivable that, due to its urinary tract tropism, inulin might find wider application in medicine as a drug carrier, especially of drugs to cure urogenital diseases. Therefore, new sources of this natural product with distinct degree of polymerization will surely be welcome.

EXPERIMENTAL

Material and Methods

The roots of the medicinal plant were purchased from the Faculty of Pharmacy, Comenius University, Bratislava. Inulin was a commercial product (Sigma). HPGPC of the fructan was performed using a

commercial instrument (Laboratorni pristroje, Prague, Czech Republic) equipped with two Tessek Separon HEMA BIO-100 exclusion columns (8 × 250 mm) and aqueous 0.1 M $NaNO_3$ as solvent (0.4 ml min^{-1}). The eluate was monitored by an IR detector. A set of pullulan standards (Shodex Standard, P-82, Macherey–Nagel, Germany) was used for calibration of the column.

The crude polysaccharide was hydrolyzed with 2 M trifluoroacetic acid at 120 °C for 2 h and the fructan with 2% oxalic acid at 100 °C for 1 h and with 0.05 M HCl at 55 °C for 2 h. The ratio of fructose to glucose in the hydrolyzate of the fructan was determined by HPLC using a Waters liquid chromatograph equipped with a Tessek Ostion LG KS 0800 column (250 × 8 mm) maintained at 90 °C. Milli Q water was used as an eluent at a flow rate of 1 ml min^{-1}. Detection was accomplished using a differential refractive-index detector (R 403, Waters Association). Peak areas were measured with a Waters model 991 integrator-recorder.

Methylation of the fructan was performed using the method of Ciucanu and Kerek[6], followed by the method of Purdie[7]. The product was converted to partially methylated alditol acetates by hydrolysis with 90% formic acid (1 h, 100 °C), then with 1 M trifluoroacetic acid (4 h, 100 °C), reduction with $NaBD_4$, acetylation, and then subjected to GC-MS analysis, effected on a FINNIGAN MAT SSQ 710 spectrometer equipped with an SP 2330 column (0.25 mm × 30 m) at 80–240 °C (6 °C min^{-1}), 70 eV, 200 μA, and ion-source temperature 150 °C.

NMR spectra were recorded at 25 °C on an FT NMR Bruker AVANCE DPX 300 spectrometer (300.13 MHz) equipped with a gradient enhanced spectroscopy kit (GRASP) for generation of *z*-gradients up to 50 gauss cm^{-1} in 5 mm inverse probe. Samples were dissolved in deuterium oxide and chemical shifts of the signals were referenced to external acetone (δ 2.225 and 31.07 for ^1H and ^{13}C, respectively). The other methods used were as described in ref.[1].

Isolation of the Polysaccharide

The roots, cut into sections and air-dried (300 g), were macerated in distilled water (18 l) for 48 h at room temperature. The suspension was then filtered, the filtrate was centrifuged, concentrated to 1.5 l, and the solution was added dropwise to ethanol (6 l, acidified with 1 vol.% acetic acid). The precipitate was collected by centrifugation, washed with aqueous ethanol (80 vol.%), centrifuged, suspended in distilled water, exhaustively dialyzed, and freeze-dried to give a brownish product (8.2 g). The crude product was loaded onto a column (5 × 120 cm) of DEAE Sephadex A-50 (carbonate form) and eluted successively with water and ammonium carbonate solutions (0.1, 0.25, and 0.5 M). The carbonate solutions were treated with Dowex 50 Wx4 (H^+), dialyzed, and freeze-dried. They were not studied further. The water eluate, making 35% of all fractions, was further separated on a column (4 × 150 cm) of Sephadex G-50. The most abundant fraction (*ca* 85%), containing fructose as the dominant sugar component, was purified on a column (2.5 × 150 cm) of Biogel P-2 to give a polysaccharide composed of fructose and glucose only. It was homogenenous by free-boundary electrophoresis and on HPGPC gave one narrow symmetrical band. This product was subjected to structure analysis.

RESULTS AND DISCUSSION

Cold-water extraction of the roots, followed by precipitation with ethanol, gave a crude polysaccharide in 2.6% yield (dry weight basis). It contained, besides carbohydrates, also proteins (8.8%) and incombustible salts (28%). The sugar components were represented by fructose, galactose, glucose, mannose, arabinose, xylose, rhamnose, and uronic acids. Acid components, pigments, and dyes were removed from the crude pro-

duct by ion-exchange chromatography, yielding a slightly coloured material. Upon hydrolysis this material showed a major spot for fructose and smaller amounts of galactose, glucose, arabinose, and rhamnose. The dominant neutral polysaccharide component was separated from the mixture by gel filtration. On HPGPC it displayed one narrow symmetrical band, corresponding to *Mn* 4 600 (DP ≈ 28, pullulan standards). Complete hydrolysis to fructose and glucose was achieved at very mild conditions, suggesting that the fructosyl residues were present in the furanose form. Examination of the hydrolyzates by HPLC showed that the fructose to glucose ratio was 26 : 1. The polysaccharide was nonreducing to Fehling's solution and had $[\alpha]_D$ –38° which is in the range observed for polysaccharides that contain mainly β-D-fructofuranosyl residues[8].

The results of methylation analysis are presented in Table I. On reduction of partially methylated D-fructoses both the D-glucitol and D-mannitol derivatives are formed. Because 1,3,4- and 3,4,6-tri-*O*-methyl-D-fructose give, in addition to partially methylated glucitol acetates, the same partially methylated mannitol acetate, the hydrolyzate of the methylated fructan was treated with $NaBD_4$ which made possible to identify [5-^{2}H]-2,5,6-tri-*O*-acetyl-1,3,4-tri-*O*-methyl-D-mannitol, generated from reduction and acetyl-

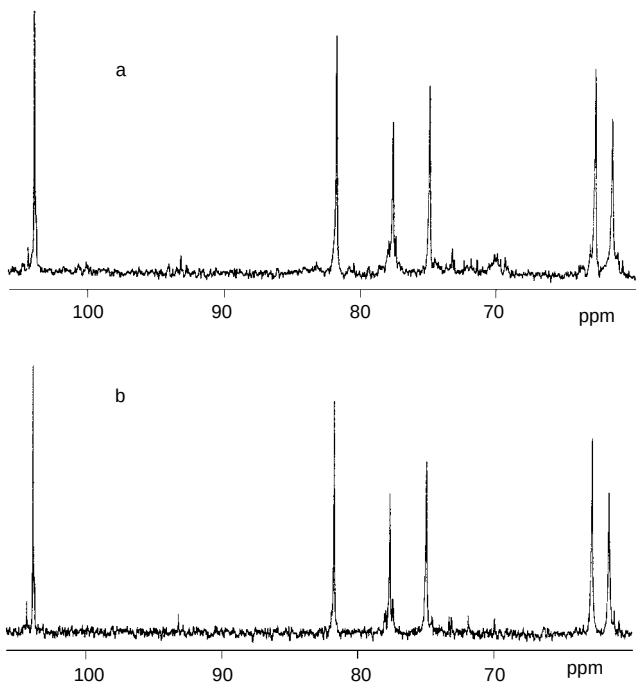


FIG. 1
^{13}C NMR spectra (75.46 MHz) with suppressed NOE of: a Fructofuranan from the roots of *Rudbeckia fulgida*, b Inulin, commercial product

ation of 3,4,6-tri-*O*-methyl-D-fructose. The derivatives in the table indicate a linear structure of the polymer with D-glucopyranosyl end-group, in accordance with the generally accepted role of sucrose-terminated β-D-fructosides in carbohydrate metabolism, in which sucrose is the primer onto which successive fructosyl groups are attached. The 3,4,6-trimethylfructose derivative evidenced that the chain fructosyl units are linked by (2→1) linkages, the end-group of the chain being nonreducing, as indicated by 1,3,4,6-tetra-*O*-methyl-D-fructose. The degree of polymerization (*DP* = 28) calculated from the mole proportions of the derivatives agreed well with that found on HPLC (*DP* = 27).

To support the results obtained by compositional and linkage analyses, we recorded the ^{1}H and ^{13}C NMR spectra of the fructan and, for comparison, the spectra of commercial inulin. The ^{13}C NMR spectra are presented in Fig. 1 and the HMQC spectra in Fig. 2,

TABLE I
Methylation analysis data of the fructofuranan

Derivative[a]	Mole ratio	Linkage indicated
2,3,4,6-Me_4-Glc	0.1	Glcp-(1→
1,3,4,6-Me_4-Fru	0.1	Fruf-(2→
3,4,6-Me_3-Fru	2.6	→1)-Fruf-(2→

[a] 2,3,4,6-Me_4-Glc is 2,3,4,6-tetra-*O*-methyl-D-glucose, *etc*., determined as partially methylated alditol acetates.

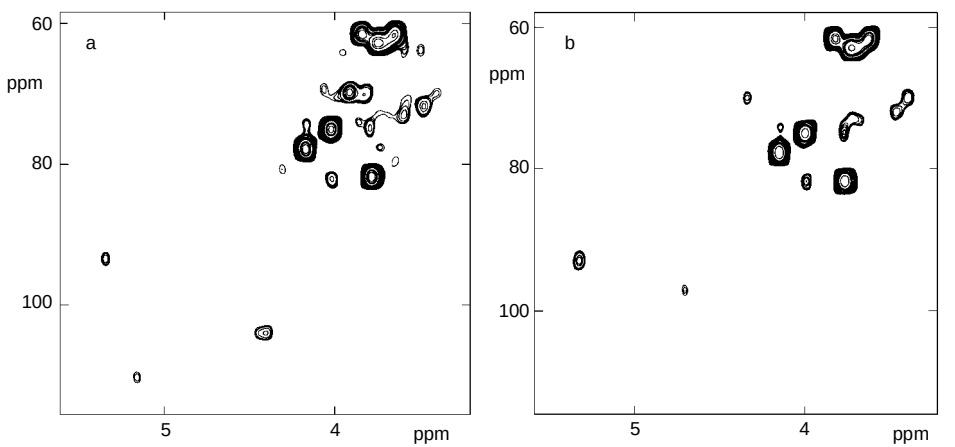
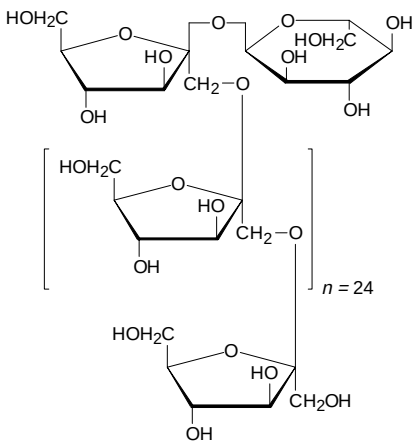


FIG. 2
HMQC spectra of: a Fructofuranan from the roots of *Rudbeckia fulgida*, b Inulin, commercial product

from which the similarity of both compounds is evident. Chemical shifts of the predominant signals due to β-D-fructofuranosyl residues are identical in both spectra, proving the simple linear structure of (2→1)-linked fructofuranan. The additional signals of very low intensity in the spectra of the fructofuranan reflected the presence of small amount (≈5%) of the residual accompanying polysaccharide species. The degree of polymerization, *DP* = 27, was estimated from the HMQC spectrum on the basis of the integral intensities of αGlc H4,C4 (δ 3.36, 70.1) and Fru H4,C4 (δ 3.99, 75.1) cross-peaks. This value was in accord with that (*DP* = 28) obtained from the C-2 signal intensities of chain fructosyl residues (δ 104.1) and the fructosyl moiety (δ 104.5) of sucrose in the 1D quantitative ^{13}C NMR spectrum, measured with suppression of NOE. The *DP* of the commercial inulin calculated in the same way was 21.

On the basis of the obtained results the structure of the fructofuranan studied corresponds to formula **1**.



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This investigation was supported by the Grants No. 2/1240 and 2/1234 of the Slovak Scientific Grant Agency (VEGA).

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