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Note

An homologous series of oligofructosides in *Arnica montana* L. roots

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Arnica montana L. (*Asteraceae*, *Compositae*) is an old remedy in folk medicine: the flowers, leaves, rhizomes, together with the roots, and the whole plant are used as a tincture, diluted with water in various proportions[1,2]. The underground parts and the flowers of *A. montana* are reported in various pharmacopoeiae[1]. Little is known of the carbohydrates of *A. montana* roots. In 1870, Dragendorff reported on the inulin content[3], and, in 1950, Thies and Herrlinger[4] demonstrated the presence of glucose, fructose, sucrose, and inulin.

During our studies of the components of *A. montana* at different stages of growth, the presence of glucose, fructose, sucrose, and a group of non-reducing oligofructosides was observed[5] in 70% ethanolic extracts of the roots. Preliminary analyses showed that these oligosaccharides belonged to a series with chromatographic behaviour similar to that of the series of oligofructosides of *Helianthus tuberosus* L tubers[6]. Homologous series of oligofructosides are widely distributed[6–8] in the underground organs of various *Asteraceae* (*Compositae*), where they play an important role in sugar metabolism, as acceptors or donors of fructosyl residues[9]. We now report on the composition and structure of the oligosaccharides present in *A. montana* roots. A comparison with the known series of oligofructosides from *Helianthus tuberosus* L. tubers, based upon statistical treatment of R_M data[10], was used to confirm the structure.

The non-reducing oligosaccharides present in *A. montana* L. roots showed the same chromatographic behaviour as those of *H. tuberosus* L. tubers and their behaviour on hydrolysis was also similar. Total hydrolysis with acid gave fructose and glucose, whereas on partial hydrolysis with acid and treatment with invertase, each oligosaccharide yielded fructose, a small proportion of glucose, and the members of the series with lower d.p.

That the oligosaccharides constituted an homologous series was shown by the regression line obtained by plotting R_M against d.p.; there was linearity for d.p. 3–6 with a correlation coefficient (r) of 0.9940. A discontinuity was found for both series of saccharides from d.p. 2 to d.p. 3.

TABLE I

R_M AND d.p. VALUES OF *Arnica montana* L. AND *Helianthus tuberosus* L. OLIGOSACCHARIDES[a]

	X_1	$(X_1)^2$	Y_1	$(Y_1)^2$	X_1Y_1	X_2	$(X_2)^2$	Y_2	$(Y_2)^2$	X_2Y_2
	0.6705	0.4496	3	9	2 0115	0.8254	0.6813	3	9	2.4762
	0.9369	0.8778	4	16	3.7476	1.1488	1.3197	4	16	4.5952
	1.1993	1.4383	5	25	5.9965	1.4342	2 0569	5	25	7.1710
	1.4432	2.0828	6	36	8.6592	1.6686	2.7842	6	36	10.0116
	1.6879	2.8490	7	49	11.8153	1.8513	3.4273	7	49	12.9591
Σ	5.9378	7.6975	25	135	32.2301	6.9283	10 2694	25	135	37 2131

[a]X_1, X_2 = R_M (average of 8–12 determinations); Y_1, Y_2 = d p.; X_1, Y_1 = values for *H. tuberosus* L ; X_2, Y_2 = values for *Arnica montana* L.

TABLE II

COMPARISON OF REGRESSION LINES

Sample	*D.f.*[a]	Σx^2	Σxy	Σy^2	*Reg. coeff.*[b]	*Deviation from regression*		
						D.f.	*S s.*[c]	*M.s.*[d]
H. tuberosus L.	4	0.6458	2.5411	10	3.9348	3	0.0013	0 0004
A. montana L.	4	0.6689	2 5716	10	3.8445	3	0.1134	0 0378
						6	0.1147	0.0191
Pooled	8	1.3147	5.1127	20	3.8889	7	0.1174	0.0168
Difference between slopes						1	0.0027	0.0027
Comparison of slopes[e]: F = 0.0027/0.0191 = 0.1415 (d.f. = 1/6); N s.								

[a]D.f. = degrees of freedom; $\Sigma x^2 = \Sigma X^2 - C$, where $C = \bar{X}\Sigma X$; $\Sigma y^2 = \Sigma Y^2 - C$, where $C = \bar{Y}\Sigma Y$; $\Sigma xy = \Sigma XY - C$, where $C = \bar{X}\Sigma Y = \bar{Y}\Sigma X$. [b]Reg. coeff. = b = $\Sigma xy/\Sigma x^2$. [c]S s. = sum of squares = $\Sigma y^2 - (\Sigma xy)^2/\Sigma x^2$. [d]M.s. = mean square = S.s /d.f. [e]F = ratio between mean squares; N.s. = non-significant.

A comparison, using the Snedecor and Cochran procedure, of the regression lines for the series of saccharides from *A. montana* and from *H. tuberosus* showed that they were parallel with a non-significant difference in slope (Tables I and II). Thus, it may be concluded that the two homologous series are formed by attachment of the same monomer unit, at the same position and with the same anomeric configuration[11], and the *A. montana* saccharides can be assigned the structure [Glc–Fru]Fru_n, in which fructofuranosyl residues are attached by (2→1′)-β linkages to the fructosyl unit of sucrose.

In samples of *A. montana* taken before flowering, the first members of a parallel series of reducing oligofructosides were also detected. These saccharides, which were reactive to triphenyltetrazolium chloride, are seemingly identical with the reducing (2→1′)-β-linked components of the series obtained by partial hydrolysis of inulin with acid[12]. Further studies are in progress.

EXPERIMENTAL

The following wild specimens were taken in the Western Alps: *Arnica montana* L. in S. Giacomo di Roburent (Cuneo), altitude 1600 m (August 1978); *Helianthus tuberosus* L. in Busca (Cuneo), altitude 500 m (September 1978).

Reference sugars were obtained from commercial sources; 2 μl of 0.5% solutions in 70% ethanol were used for t.l.c. The oligosaccharides were extracted from the underground parts of the plants with hot 70% ethanol. T.l.c. was performed[10] on Kieselgel G (Merck) with chloroform–acetic acid–water (3:3.5:0.5, 3–5 developments at 28°). The oligosaccharides were non-reactive to triphenyltetrazolium chloride, but were detected by using diphenylamine–aniline phosphate

Acid and enzymic hydrolyses were performed by the "*in situ*" technique[13] on 5-μl portions of the ethanol extract (corresponding to 6.25 mg of fresh material), spotted on the Kieselgel G layer; multiple developments were used in each direction to give a two-dimensional chromatogram.

Partial and total hydrolyses were performed, respectively, with 25mM and 0.25M hydrochloric acid, at 80–100° for 30 min. Enzymic hydrolysis was performed with a 0.01% aqueous solution of β-D-fructosidase (Boehringer, from yeast) at 37° for 30 min.

For each component, mean R_M values[14] were calculated from hR_{Glc} values (8–12 determinations): $hR_{Glc} = 100\,R_{Glc}$; $R_M = \log(1/hR_{Glc} - 1)$. For R_{Glc}, single-ascent values were used[11,15]. Plots[15] of R_M against d.p. were made for the two series of saccharides. The slopes of the regression lines were compared by using the procedure of Snedecor and Cochran[16].

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