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Note

A homologous series of non-reducing oligosaccharides in *Artemisia absinthium* L. roots

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The structure and metabolism of a series of oligofructosides in *Helianthus tuberosus* L. tubers was reported by Dedonder[1] and by Edelman and co-workers[2,3]. These components, formed through transfer of fructosyl residues on a terminal sucrose unit, are widely distributed in plant tissues where they represent a fraction metabolically connected with sucrose and fructosans[4].

In a previous investigation of the soluble carbohydrates present in roots of various *Asteraceae*, a group of non-reducing oligofructosides was identified in *Artemisia dracunculus* L. roots[5]. Preliminary studies demonstrated that these saccharides belong to a continuous homologous series, at least up to a degree of polymerization (DP) of 6–8.

In the course of further studies, reported in the present paper, the series of non-reducing oligosaccharides present in *Artemisia absinthium* L. roots was examined. Chromatographic separation on silica gel thin layers and "*in situ*" acid and enzymatic hydrolysis were used in order to elucidate the composition of the oligosaccharides; comparison with the known series of oligofructosides from *Helianthus tuberosus* L. tubers, based upon statistical treatment of R_M data for the two series, was applied to confirm the structure.

EXPERIMENTAL

The underground parts from the following wild specimens were taken in September 1978 in the West Alpine region: *Artemisia absinthium* L. in Usseaux (Torino), altitude 1400 m; *Helianthus tuberosus* L. in Busca (Cuneo), altitude 500 m. Reference sugars were obtained from commercial sources; 2 μl of 0.5% sample solutions in 70% ethanol were spotted on the plate.

The oligosaccharides, extracted from roots and tubers with hot 70% ethanol, were fractionated on Kieselgel G (E. Merck, Darmstadt, G.F.R.) layers with chloroform–acetic acid–water (3:3.5:0.5)[6] (three ascents at 28°C); 10 μl, corresponding to 20 mg of fresh material, were spotted on the plate. The spots, non-reactive to triphenyltetrazolium chloride (TTC)[7], were detected with the spray reagent diphenylamine aniline phosphate (DAP)[8].

Acid and enzymatic hydrolyses were performed with the "*in situ*" technique[9] on 5 μl of ethanol extract spotted on a Kieselgel G standard layer; the same eluent

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(see above) was used in both directions. The multiple-ascents technique was employed for better resolution.

Partial and total acid hydrolyses were performed respectively with 0.025 and 0.25 *N* hydrochloric acid, at 80–100°C for 30 min. Enzymatic hydrolysis was carried out with a 0.01% aqueous solution of β-fructosidase (from yeast; Boehringer, Mannheim, G.F.R.), at 37°C for 30 min.

For each component, hR_G and R_M values were calculated*. Graphs of R_M against DP (degree of polymerization) were drawn[12] using the data obtained for the two series of saccharides. The comparison of the slopes of regression lines was performed according to Snedecor and Cochran[13].

RESULTS AND DISCUSSION

The chromatographic behaviour of the non-reducing oligosaccharides detected in *A. absinthium* L. roots was comparable with that observed for the non-reducing oligosaccharides of *H. tuberosus* L. tubers.

On total acid hydrolysis, each component gave only fructose and glucose. On partial acid and invertase hydrolysis, each oligosaccharide yielded fructose, a small amount of glucose and the members with lower DP (see Fig. 1); *e.g.*, from the trisaccharide, sucrose was formed.

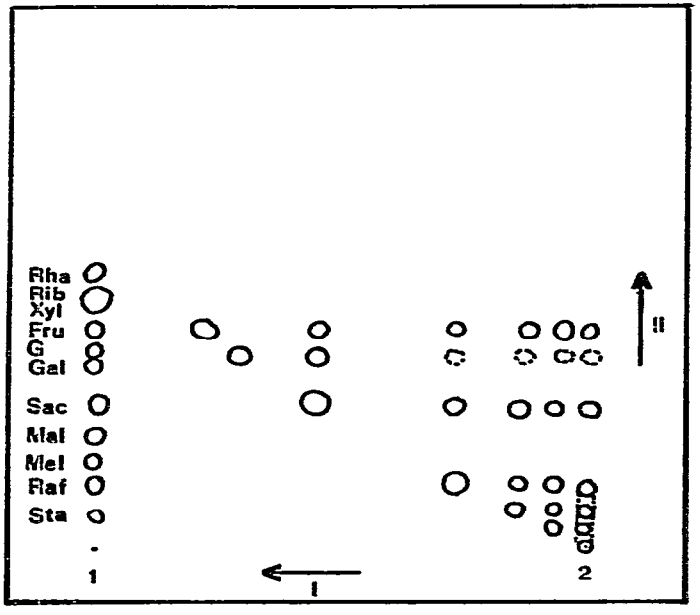

Fig. 1. "*In situ*" partial acid hydrolysis of *A. absinthium* L. saccharides on Kieselgel G. Solvent system: chloroform–acetic acid–water (3:3,5:0,5); three ascents at 28°C in each direction. Spray reagent: diphenylamine aniline phosphate. Samples: 1, sugars (rhamnose, ribose, xylose, fructose, glucose, galactose, saccharose, maltose, melibiose, raffinose and stachyose); 2), *A. absinthium* L. ethanol extract.

* $hR_G = R_G \cdot 100$; $R_M = \log (1/hR_G - 1)$ (ref. 10). For R_G, *i.e.*, R_F relative to glucose, single-ascent values were used[11,12].

The regression line obtained by plotting R_M against DP for the oligosaccharides with DP from 3 to 6 showed linearity* with a correlation coefficient $r = 0.9791$. This indicates that these members fall into a continuous homologous series.

The regression lines for the series of saccharides from *A. absinthium* L. roots and for the known series extracted from *H. tuberosus* L. tubers, compared through the Snedecor and Cochran procedure, appeared to be parallel with a non-significant difference in slope (see Tables I and II); this suggests that the two groups of saccharides

TABLE I

R_M AND DP VALUES OF *ARTEMISIA ABSINTHIUM* L. AND *HELIANTHUS TUBEROSUS* L. OLIGOSACCHARIDES

X_1, $X_2 = R_M$ (average of 8–12 data); Y_1, Y_2 = DP. X_1, Y_1 = values for *H. tuberosus* L., X_2, Y_2 = values for *A. absinthium* L. $\Sigma x^2 = \Sigma X^2 - C$, where $C = \bar{X}\Sigma X$; $\Sigma y^2 = \Sigma Y - C$, where $C = \bar{Y}\Sigma Y$; $\Sigma xy = \Sigma XY - C$, where $C = \bar{X}\Sigma Y = \bar{Y}\Sigma X$.

	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	0.7279	0.5298	3	9	2.1837
	0.9369	0.8778	4	16	3.7476	1.0373	1.0760	4	16	4.1492
	1.1993	1.4383	5	25	5.9965	1.3669	1.8684	5	25	6.8345
	1.4432	2.0828	6	36	8.6592	1.4668	2.1515	6	36	8.8008
	1,6879	2.8490	7	49	11.8153					
Σ	5.9378	7.6975	25	135	32.2301	4.5989	5.6257	18	86	21.9682

Helianthus tuberosus L.

ΣX_1^2	7.6975	ΣY_1^2	135	ΣX_1Y_1	32.2301
C	7.0517	C	125	C	29.6890
Σx_1^2	0.6458	Σy_1^2	10	Σx_1y_1	2.5411

Artemisia absinthium L.

ΣX_2^2	5.6257	ΣY_2^2	86	ΣX_2Y_2	21.9682
C	5.2874	C	81	C	20.6948
Σx_2^2	0.3383	Σy_2^2	5	Σx_2y_2	1.2734

TABLE II

COMPARISON OF REGRESSION LINES

Comparison of slopes: $F = 0.0064/0.0416 = 0.1538$ (d.f. = 1/5) N.S.; d.f. = degrees of freedom; Σx^2, Σxy, Σy^2: see Table I; reg. coef. = $b = \Sigma xy/\Sigma x^2$; S.S. = sum of squares = $\Sigma y^2 - (\Sigma xy)^2/\Sigma x^2$; M.S. = mean square = S.S./d.f. F = ratio between mean squares; NS = non-significant.

Sample	*d.f.*	Σx^2	Σxy	Σy^2	*Reg. coef.*	*Deviations from regression*		
						d.f.	*S.S.*	*M.S.*
H. tuberosus L.	4	0.6458	2.5411	10	3.9348	3	0.0013	0.0004
A. absinthium L.	3	0.3383	1.2734	5	3.7641	2	0.2068	0.1034
						5	0.2081	0.0416
Pooled	7	0.9841	3.8145	15	3.8761	6	0.2145	0.0358
Difference between slopes						1	0.0064	0.0064

* On passing from the component with DP = 2 (sucrose) to that with DP = 3, a discontinuity was found for both series of saccharides.

belong to the same type of homologous series, *i.e.*, that both series are derived by attachment of the same type of monomer unit, with the same mode of attachment[11].

On the basis of these results, it can be inferred that the non-reducing oligosaccharides extracted from *A. absinthium* L. roots belong to a sucrose-terminated series, in which the added fructofuranose units are joined by $\beta(2 \rightarrow 1')$ linkages to the fructose unit of sucrose. The β-fructofuranose structure is also consistent with the enzymatic hydrolysis.

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