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Monosaccharides of the Green Fresh-Water Alga *Chlorella kessleri*

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ABSTRACT. Both qualitative and quantitative representation of monosaccharides of autotrophically and heterotrophically cultivated alga *Chlorella kessleri* was determined. Polysaccharides obtained after separation of lipids from the alga were subjected to amylolysis and hydrolysis. Individual monosaccharides were determined in the form of per-O-acetylalditols using the method of gas chromatography–mass spectrometry.

Green fresh-water algae do not contain as many various polysaccharides as do marine algae (Percival and McDowell 1981). This fact is reflected in the low number of studies concerned with the structure of polysaccharides of green algae; one of them is the paper by Olaitan and Northcote (1962) concerned with the structure of polysaccharides of *C. pyrenoidosa*. For instance, a trisaccharide consisting of galactose, arabinose and rhamnose was isolated from them. Papers, in which the representation of monosaccharides was determined after hydrolysis of polysaccharides, are more frequent. Thus, in the genera *Chlorella* and *Scenedesmus*, glucose, mannose, galactose, arabinose, xylose, rhamnose and fucose were found by Northcote *et al.* (1958) and by Shamala *et al.* (1982).

As far as methods are concerned, paper chromatography (Olaitan and Northcote 1962; Northcote *et al.* 1958) or thin-layer chromatography (Becker and Shefner 1964) are most frequently used. Recently, gas chromatography (Dutton 1973, 1974) and mainly gas chromatography–mass spectrometry (GC–MS), when monosaccharides can be identified by their retention characteristics and mass spectra (Lönngren and Svensson 1974), predominate.

Chlorella kessleri appears to be most interesting as a source of unusual secondary metabolites (Řezanka *et al.* 1983). The representation of monosaccharides in polysaccharides of this alga was studied by the GC–MS method.

MATERIALS AND METHODS

The alga *Chlorella kessleri* was provided by the *Department of Autotrophic Microorganisms* of the Institute of Microbiology, Czechoslovak Academy of Sciences (Třeboň, Czechoslovakia). From 10 g of algal material 52 mg of

polysaccharides I (autotrophic cultivation) and 65 mg of polysaccharides II (heterotrophic cultivation) were prepared according to Olaitan and Northcote (1962). 47 mg of polysaccharides I or 51 mg of polysaccharides II was dissolved in 10 mL acetate buffer (0.2 M, pH 7) and 10 mg of exo-1,4-α-D--glucosidase (EC 3.2.1.3) (Sigma, GFR) was added. After 20 h at 36 °C the mixture was heated to 100 °C, the denatured enzyme was centrifuged (30 min, 2 500 *g*) and the mixture was desalted on the cationic ion exchanger Dowex 50 in a H^+ cycle. The resulting solution was evaporated to dryness (in order to remove acetic acid), dissolved in 5 mL water and precipitated with 50 mL ethanol. After centrifugation 9.8 mg polysaccharides III (from polysaccharides I) or 6.8 mg polysaccharides IV (from polysaccharides II) was obtained. The polysaccharides were hydrolyzed according to Olaitan and Northcote (1962) and reduced according to Oades (1968). The yield of per-O-acetylalditols from polysaccharides III was 12.3 mg, from polysaccharides IV it was 7.1 mg.

The GC—MC measurements were performed in a Hewlett-Packard 5985B (USA) equipped with a glass WCOT capillary column ID 0.25 mm × 30 m (N_{eff}/m = 2120) with the stationary phase SP-2330 (Supelco, USA), split ratio 100 : 1, sample size 1 μL. Working regime: ionization energy 11 aJ (70 eV), scan speed 690 m_u/s, mass range 4-600 m_u, programmed temperature from 210 to 230 °C at a rate of 1 K/min and further isothermically, injector temperature 250 °C, helium flow 1.1 mL/min.

Standards of per-O-acetylalditols were prepared from the corresponding sugars (glucose, mannose, galactose, arabinose, xylose, rhamnose, Lachema Czechoslovakia; fucose, 2-deoxyribose, glucuronic and galacturonic acids, Sigma GFR) according to Oades (1967).

RESULTS AND DISCUSSION

For the GC of saccharides their per-O-trimethylsilyl derivatives are most commonly used. As their cyclic form remains preserved anomerization occurs at $C_{(1)}$ and monosaccharides thus yield two peaks (anomers α and β), which, due to a high number of components, make the identification more difficult. It is hence more useful to reduce the monosaccharides to alditols and chromatograph these compounds after their derivatization. Per-O-acetylalditols are separated both on packed columns with highly polar phases ECNSS-M (Oades 1967), EGSS-X and HiEFF-1BP (Lehndardt and Winzler 1968) and on capillary columns with phases OV-275 (Klok *et al.* 1981), Silar 10C (Shibuya 1981) and chemically bound stationary phase BP-75 (Blakeney *et al.* 1982). We were satisfied with the phase SP-2330 which corresponds to the above phases on capillary columns according to its parameters (McReynolds' constants). For instance on the WCOT capillary column with the phase SP-2330 all components of the mixture were remarkably well separated (Fig. 1).

Table I presents the results of both qualitative and quantitative analysis of monosaccharides in polysaccharides produced by *C. kessleri*. It is known from the literature (Olaitan and Northcote 1962; Takeda and Hirokawa 1978) that green algae contain starch as the major polysaccharide.

Therefore, samples for analysis were first treated by amylolysis as a selective method leading to the removal of starch. The generally used α-amylase (EC 3.2.1.1) is not particularly useful as it does not degrade 1→6 bonds, and, thus, oligosaccharides that are difficult to remove remain in the solution.

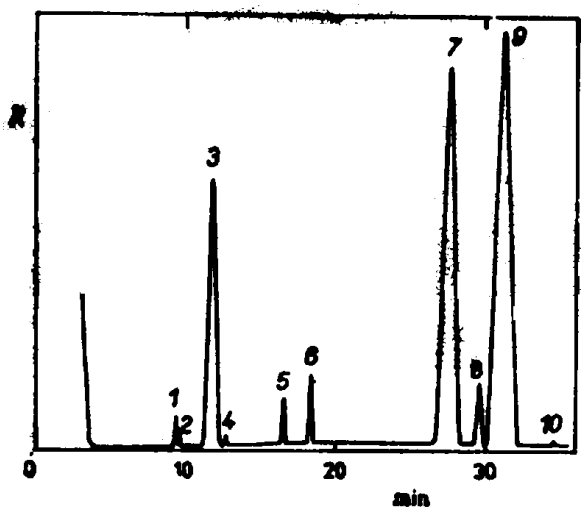

FIG. 1. Gas chromatography of monosaccharides (*cf.* Table 1) after hydrolysis of the polysaccharide of *C. kessleri* (autotrophic cultivation); *R* response of the detector.

This disadvantage is avoided when using exo-1,4-α-D-glucosidase which degrades starch all the way to glucose (it degrades both 1 → 4 and 1 → 6 bonds).

Monosaccharides corresponding to peaks 3 and 5—9 are commonly detected in green algae (Northcote *et al.* 1958; Becker and Shefner 1964), whereas fucose was found only in the genus *Scenedesmus* (Shamala *et al.* 1982). Deoxypentoses as components of natural polysaccharides have not yet been described. When 2-deoxyribose is not considered only the antibiotically active 1-deoxy-D-*threo*-pentulose was detected as a natural deoxypentose (Hoeksema and Baczynskyj 1976). Its structure was verified by mass, IR and ^{1}H-HMR spectra. The preliminary structure of two deoxypentoses detected by us (5-deoxy- and 2- or 4-deoxypentoses) is based on mass spectra, the 2(4)-deoxypentose not being identical with 2-deoxyribose (Table I). On the basis of mass spectra it cannot be determined whether 2- or 4-deoxypentose is involved as a primary alcohol group is formed by reduction even at the other end of the molecule.

TABLE I. Content of monosaccharides in polysaccharides of *C. kessleri*

Peak No.	Monosaccharide[a]	RRT[b]	Polysaccharides III %	Polysaccharides IV %
1	5-deoxypentose	0.57	2.2	2.7
1	2(4)-deoxypentose	0.56[c]	0.7	0.3
3	rhamnose	0.71	18.2	16.2
4	fucose	0.77	0.8	1.8
5	arabinose	1.00	3.3	12.8
6	xylose	1.18	5.1	14.1
7	mannose	1.67	31.4	35.7
8	galactose	1.79	4.5	13.1
9	glucose	1.89	33.4	3.0
10	galacturonic acid	2.05	0.4	0.3

[a] Chromatographed as per-O-acetylalditols on the WCOT column with the phase SP-2330.
[b] Relative retention time as related to penta-O-acetylarabinitol (retention time 16.5 min).
[c] RRT of 2-deoxyribose 0.60.

The presence of galacturonic acid is also remarkable as its occurrence is common only in polysaccharides of marine algae (Percival and McDowell 1981). In *C. ellipsoides* and *C. vulgaris* only glucuronic acid was detected to be a component of the extracellular polysaccharide (Moore and Tischer 1964). On the other hand, we could not demonstrate glucosamine detected in high amounts by Takeda and Hirokawa (1979).

It follows from the percent representation of individual monosaccharides (Table I) that the content of 5-deoxypentose, rhamnose and galacturonic acid remains the same, irrespective of the type of cultivation. On the other hand, during autotrophic cultivation the content of 2(4)-deoxypentose and glucose increases, that of the latter compound by more than 11 times. On the contrary, the content of fucose, arabinose, xylose and galactose is higher during heterotrophic cultivation, roughly 2-4-fold in any of the sugars. The effect of cultivation conditions on the content of monosaccharides, glucose in particular, is explained by the course of photosynthesis in autotrophy: glucose is first synthesized and other monosaccharides are then produced from it whereas under heterotrophic conditions it is degraded and sugars are synthesized from simple precursors, although glucose served as a carbon source.

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