DETERMINATION OF THE AMOUNT OF MANNITOL IN BROWN SEAWEEDS

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One of the main components of brown seaweeds is mannitol [1], which is used in industry and medicine [2-5]. One of the numerous existing methods of determining the amount of mannitol, the one used most frequently, is colorimetry in the presence of chromotropic acid [6-1]]. However, it possesses serious defects [12-15].

We have investigated the possibility of using methods that we have developed previously for determining formaldehyde [16-17] for the analysis of mannitol in brown seaweeds.

The first stage in the determination of the polyol is its extraction from the plant by the most suitable solvent. Various solvents and mixtures of them have been used for the extraction of mannitol from seaweeds (1, 18-21]. A comparative test of extraction with methanol, acetone, 50% aqueous isopropanol, 10% aqueous butanol, and butanol performed on the seaweed Sargassum by determining the amount of extractable substances and finding their composition by thin-layer chromatography showed that the best solvent is aqueous isopropanol. Three or four extractions of 10-15 min each are sufficient to extract the mannitol completely. The other compounds present together with mannitol in brown seaweeds (alginic acid, laminarin, laminitol, phycosterol, pelvesterol, terpenes, proteins, higher and lower fatty acids, and others [1, 22-25]) do not interfere with its determination. Thus, the amount of mannitol can be determined directly in the isopropanol extract.

TABLE 1

Weight of sample, g	Mannitol content (%) obtained by			
	the phenylhydrazine method		the acetylacetone method	
	crude wt.	dry wt.	crude wt.	dry wt.
0,6025	4,8	12,0	4,6	11,5
0,7009	4,8	11,9	4,4	10,9
0,6883	4,3	10,7	4,3	10,7
0,5056	4,7	11,9	4,4	11,1
Mean value	4,6	11,6	4,4	11,1
Maximum deviation from the mean	+0,2 −0,3	+0,4 −0,9	+0,2 −0,1	+0,4 −0,4

To check the reproducibility of the results obtained by the phenylhydrazine and acetylacetone methods, four samples taken from one and the same position of a laminaria frond were analyzed in parallel. The mean values obtained by the two methods agreed well (11.6% and 11.1%) (Table 1). We have previously checked the accuracy of both methods with a standard solution of mannitol [16, 17].

TABLE 2

Seaweed	Mannitol content (% on the dry wt.) determined by	
	phenylhydrazine method	acetylacetone method
Laminaria cychoriodes	20,7	21,0
Sphaerotrichia sp.	4,1	4,9
Dyctiota sp.	7,3	7,8

The two methods also gave results which agreed well in the comparative determination of mannitol in various seaweeds (Table 2). An advantage of the phenylhydrazine method is that the measurements can be performed with satisfactory accuracy on a photoelectric colorimeter, while for the acetylacetone method a spectrophotometer is necessary, Consequently, the seaweeds were subsequently analyzed by the phenylhydrazine method.

To determine their dry weight, the seaweeds were dried in a thermostat at 100° C.

Then we investigated how the mannitol is distributed over the thallus of a large seaweed—laminaria. At different parts

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TABLE 3

Samples	Mannitol content, %	
	in crude seaweed	calc. to dry wt.
Rhizoids	0,6	3,8
Pedicel	1,1	7,9
Points of the lamina*		
3rd	2,6	21,9
4th	4,7	26,8
5th	5,3	33,2

*In zone 6 the mannitol content was not determined since, as a rule, this part of the lamina is highly broken down.

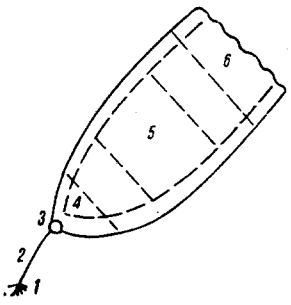

Fig. 1. Parts of a seaweed: 1) rhizoids; 2) pedicel; 3, 4, 5, 6) points of the lamina.

of the seaweed, the amount of mannitol both on the crude weight* and on the dry weight varies sharply (by a factor of about 10) (Table 3; Fig. 1). Consequently, for a comparative analysis of different seaweeds samples must be taken from strictly corresponding parts of them or a mean sample from a mixture of different parts must be used.

Fourteen different species of seaweed belonging to six orders were examined by this method (Table 4). The figures of Table 4 show a well-defined correlation between the mannitol content and the systematic position of the seaweed. It was confirmed once more that in the most industrially useful seaweeds – the laminaria – the mannitol content may be very high [26, 23].

Thus, the methods that we have developed for determining formaldehyde can be applied to the analysis of the mannitol content in the raw material, to monitoring the production process, and to determining the dependence of the composition of seaweeds on various conditions of life, stages of development, etc.

EXPERIMENTAL

The following commercial reagents were used for the experiments: methanol, butanol, isopropanol, and acetone. The other reagents were prepared by published methods [16, 17]. The work was carried out in Pos'et and Kamenka bays (shore of the Sea of Japan) in June-August. All the analyses were performed on freshly collected seaweeds.† The conditions of the analysis (determination of the time and amount of solvent necessary for complete extraction of the mannitol) were worked out with samples of Laminaria cychorioides. The optical densities were measured on an SF-4A spectrophotometer at 412 and 520 nm and an FÉK-M photoelectric colorimeter (with a green filter). In all cases where it is not mentioned specifically, the ratios of the components in the mixtures of solvents are given by volume.

Determination of the Optimum Extraction Conditions. A weighed sample of seaweed (~250 mg) in dense tissue (7 × 7 cm), previously boiled in a solvent until the phenol reaction of polysaccharides was negative and dried, was placed in a flask and covered with 20 ml of isopropanol–water (1 : 1) and the mixture was boiled in the water bath. After 10, 20, 30, and 60 min, aliquots were taken, and the amount of polyol in them was determined by the phenylhydrazine method. A standard sample of tissue extracted in parallel with the samples of seaweeds was used as control.

The aliquots taken were analyzed in graduated test tubes (10 ml), the amounts of all the reagents being reduced by a factor of 2.5 in comparison with the published method [16]. After extraction for 60 min, the solution was poured off, and the sample was covered with a new portion of extraction mixture (20 ml) and heated. This operation was repeated until identical optical-density readings were obtained for the solution being analyzed and for the control.

Influence of Products Present in the Extracts on the Color Reaction. The substances present in the extracts together with the mannitol were isolated by preparative thin-layer chromatography. The behavior of these substances when they were oxidized with sodium metaperiodate ($NaIO_4$) was evaluated by the formation of a colored complex with acetylacetone.

Check of the Accuracy of the Phenylhydrazine and Acetylacetone Methods. The mannitol contents were determined in four accurately weighed samples from the same point of a laminaria frond. The same thing was done with other seaweeds (see Table 2).

Influence of the Methods of Drying on the Results of the Determination of the Mannitol Content. Laminaria cychorioides was used as the sample. Accurately weighed samples from the comminuted thallus

*Weight of the freshly collected seaweed freed from superfluous water with filter paper.

† The seaweeds were identified by members of V. L. Komarov Botanical Institute (Yu. E. Petrov and L. N. Perestenko).

TABLE 4

Seaweed	Mannitol content, %		Seaweed	Mannitol content, %	
	crude wt.	dry wt.		crude wt.	dry wt.
Class Phaecaporeae			Laminariales		
Order Chordariales			Laminaria japonica	5,3	33,2
Leathesia diffornlis	0,05	3,7	Laminaria cychorioides	4,2	20,7
Sphaerotrichia divaricata	0,6	4,1	Costaria costata	2,6	13,8
Chordaria magelanica	0,6	3,0	Class Cyclosporeae		
Heterochordaria abienita	1,3	9,2	Fucales		
Desmareatiales			Fucus evanescens	1,7	11,5
Desmarestia viridis	0,4	5,8	Pelvetia wrightii	1,0	5,5
Dictyotales			Sargassum pallidum	1,8	17,4
Dictyota dichotoma	0,9	7,3	Cystoseira crassipes	1,1	6,3
Scytosiphonales					
Scytosiphon lomentarius	0,9	8,7			

Note. The samples were taken from all parts of the seaweed or, in the case of Laminaria japonica, from the middle part of the thallus.

of the laminaria were taken, and they were dried to constant weight in a vacuum desiccator over P_2O_5 and in a drying chest at 100° C (three samples for each determination). After drying, the samples were extracted, and the mannitol contents in the solutions were determined in parallel by the two methods.

Distribution of Mannitol in the Different Parts of a Seaweed. The distribution of the substances in the thallus of Laminaria japonica was determined. Two samples for the determination of mannitol by the phenylhydrazine method were taken from each zone together with a sample for drying to calculate the mannitol content on the dry matter.

Thus, the procedure for the determination of mannitol in seaweeds is as follows: an accurately weighed sample of the freshly collected seaweed is extracted with 50% aqueous isopropanol (4 × 20 ml). The time of each extraction is 10-15 min. All the extracts are collected in a 250-ml measuring cylinder and are made up to the mark with water; aliquots are taken and analyzed for their mannitol content by the phenylhydrazine or the acetylacetone method; in parallel, two weighed samples are taken from the same seaweed, and after their crude weight has been determined they are dried to constant weight in the drying chest at 100° C; the results of the determinations are calculated to crude and dry weights.

SUMMARY

A method has been developed for determining the mannitol content in brown seaweeds. A quantitative analysis of the mannitol in the most important brown seaweeds of the Sea of Japan has been given.

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