Characterization of Starch from Marsh Mallow (*Althaea officinalis* L.)

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From the insoluble residue of the roots of marsh mallow after extraction of mucilages starch has been isolated by various methods and characterized by amylose content, retrogradation as well as by susceptibility to α-, β-, and γ-amylases. The characteristics obtained have been compared to those of the potato starch.

Plant drugs represent a wide scale of biologically active compounds, some of which have not been characterized so far. Medicinal plants have been applied in medicine mainly in the classical form of various extracts, however, utilized have been also compounds isolated from these drugs, their derivatives or modified forms with precisely defined chemical structures.

The composition of mucilages is relatively complicated, polysaccharides constituting an appreciable portion of this complex material [1—5]. Investigation of mucilages from *Althaea officinalis* L., var. *Robusta* [6] was initiated by the Research Institute of Medicinal Plants in Olomouc [7] and motivated by needs of pharmaceutical practice in treatment of catarrhs of the respiratory system [8] as well as in dermatology and cosmetics [9].

In the present paper we describe the isolation and characterization of the starch remaining in the water-insoluble residue after removal of mucilages.

EXPERIMENTAL

Dried, unpeeled roots of marsh mallow (*Althaea officinalis* L.) were crushed and extracted with cold water to obtain the active heteropolysaccharides [6]. The insoluble residue (IR) served as the starting material for isolation of starch.

The enzyme preparations were tested on a soluble starch of anal. grade (Lachema, Brno; characterized in the laboratory by the iodine test and by M_r found to be 54 000, *i.e.* DP = 33.3). Potato starch (Slovenské škrobárne, Spišská Nová Ves) was used as a standard polysaccharide.

α-Amylase from *Bacillus subtilis* (specific activity 127 nkat mg^{-1}), β-amylase from barley (41 nkat mg^{-1}), and γ-amylase (82 nkat mg^{-1}) were commercial products (Koch-Light, Colnbrook); the activities were determined at working pH of 5.8, temperature 25 °C, and substrate concentration 1 mg cm^{-3}. All chemicals used were of anal. grade.

Solid samples and the isolated portions of starch were washed several times with methanol and dried *in vacuo* at 60 °C over P_2O_5. The polysaccharide solutions were prepared by dissolving the moistured samples in 2 M-NaOH. The pastes after 24 h standing in a refrigerator were neutralized with 2 M-HCl and diluted with water or by the used buffer to the respective concentration of the substrate and salt.

In the iodine test the sample (the amount depending on the starch content so that approximately 1 % solution was formed) was suspended in distilled water (10 cm^3), boiled shortly, and cooled. Then 0.1 M-HCl (0.5 cm^3) and 0.01 M iodine solution (5 drops) were added.

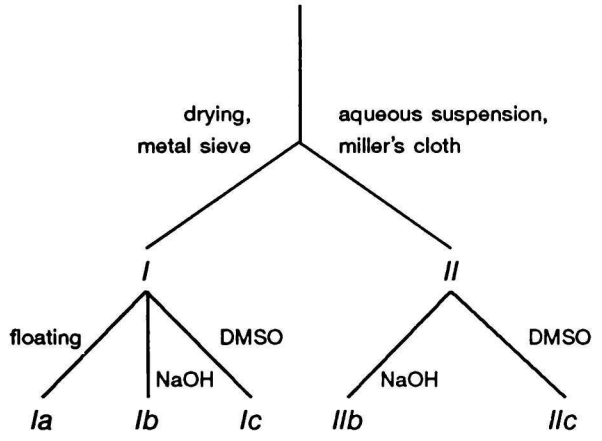
The amylose content was established from the iodine sorption biamperometrically [10]. The apparent viscosity was determined [11] at the starch concentration ρ = 2.5 g dm^{-3} in 2 M-NaOH at 25 °C by using an Ubbelohde viscometer (water value 96.2 s).

Retrogradation of starch was determined without stirring [12] of its 2.5 % solutions obtained by dissolution of the sample in 2 M-NaOH and neutralization with 2 M-HCl to pH 5.2. The aliquots withdrawn at time intervals were centrifuged and the supernatant was used to measure the iodine sorption. Retrogradation was expressed in % of the determined sorption relative to the original value.

Isolation of Starch

A. The insoluble residue (IR; 200 g) after extraction of the mucilage was dried (48 g dry material) and sieved through a metal sieve of 0.040 mm to give 16.8 g of portion *I* (Scheme 1) characterized by starch content.

B. The same amount as in *A* of IR was suspended in distilled water (1200 cm^3) and sieved



Scheme 1

Fractionation of the insoluble residue of marsh mallow roots.

in a moist state through a miller's cloth XX9. Centrifugation of the obtained suspension (1500 *g*; 15 min), washing of the sediment successively with water and methanol, repeated centrifugation (1000 *g*), and drying resulted in product *II* (9.7 g). Subsequent centrifugation of the original supernatant (1500 *g*; 45 min) yielded a brown pasty sediment (2.5 g) of low starch content.

C. The product *I* (5 g) was purified by floating to give *Ia* (2 g).

D. The products *I* and *II* (each 3 g) were further worked up by extraction with sodium hydroxide. The paste prepared by addition of 2 M-NaOH (30 cm^3) was neutralized and made up to 300 cm^3 with distilled water and centrifuged (1500 *g*). From the supernatant the starch was precipitated with the same volume of methanol. The sediment was washed and dried as usual to give *Ib* (1.6 g) and *IIb* (2.3 g).

E. The suspensions of *I* and *II* (each 3 g) in dimethyl sulfoxide (100 cm^3) were shaken in a closed vessel for 48 h at room temperature. After centrifugation (1500 *g*) the transparent solutions were precipitated with three-fold amount of methanol. Filtration and drying resulted in *Ic* (1.5 g) and *IIc* (2.0 g).

F. IR (100 g; 24 g dry mass) was suspended in distilled water (500 cm^3). Twice repeated sieving through a miller's cloth gave particles which were washed with water in a high volumetric flask and allowed to settle freely. The settled starch was dissolved in alkaline solution which was diluted, neutralized, and centrifuged (1500 *g*). Starch was precipitated from the transparent supernatant by slow addition of equal volume of methanol. The precipitate was washed with methanol repeatedly, filtered through a sintered glass filter, and dried in a desiccator over P_2O_5. Yield 5.9 g (24.59 %); the amylose content was 20.8 %.

Enzyme Hydrolysis

In α-, β-, and γ-amylolyses the enzyme solutions (according to their activities) were added into the substrate solution so that the reducing power did not exceed 10 % of its maximum value within the first 15 min (at conditions as in determination of enzyme activities).

The progress of hydrolysis was monitored photometrically by determination of the amount of reducing sugars in time intervals according to the methods of *Somogyi* [13] and *Nelson* [14], using the calibration graph for D-glucose, measured at the working pH.

The enzymes characterized by their specific activities on the soluble starch as the substrate were used also in hydrolysis of potato starch which served as a reference substrate for the marsh mallow starch.

RESULTS AND DISCUSSION

The insoluble residue of the crushed roots of marsh mallow after extraction of the mucilage was found by the informative iodine test to contain a rather large amount of starch. As the amount of the isolated mucilage was approximately 10 % [6] and that of the starch isolated from the residue was 24.6 %, the starch content in the untreated roots was about 22 % while the rough tissue amounted to 67 %.

Starch was obtained either by washing of the suspension through a miller's cloth or by sieving the dry sample through a metal sieve. Floating of the granules as well as extraction of starch both with sodium hydroxide solution and dimethyl sulfoxide was carried out. The obtained portions were evaluated from the point of view of yield and purity and characterized by iodine sorption. The method used for determination of amylose in starches from various plant materials provided in this case relative values of starch contents in the samples. The found data are presented in Table 1 (the starch content expressed relative to that of the fraction with the highest purity).

With the material under study the least advantageous and most laborious was shown to be the method of floating the sample and sieving in a moist state, because the residual mucilage in the starting material increased the viscosity of the suspension and, consequently, brought about a decrease in the rate of flow through the sieve. In

Table 1. Characterization of Starch Fractions

Sample	Yield[a]	Starch content
	%	%
I	35.00	50.76
II	19.95	57.07
Ia	40.00	49.24
Ib	53.30	68.26
IIb	76.60	92.12
Ic	50.00	100.00
IIc	67.00	100.00

a) Yields calculated to the starting material in the respective isolation procedure or purification.

this case not only the yield but also the purity of the obtained product was lower (not presented in Table 1).

For purification of the crude preparation (obtained by sieving either in dry or moist state) dissolving in alkaline hydroxide solution or dimethyl sulfoxide and subsequent precipitation with methanol was shown to be more advantageous, since it resulted in products (*IIb*, *Ic*, *IIc*) of higher purity and higher yields. The isolation method *F*, combining the individual procedures, provided practically pure starch.

The starch isolated from marsh mallow was characterized beside its linear component also by apparent viscosity, rate of retrogradation, and enzyme susceptibility.

The found value of relative viscosity 1.58 is low when compared to the value 5.55 obtained with the reference potato starch. Obviously, it is a result of the preceding treatment of the roots in isolation of the mucilage and of the treatment in isolation and purification of the starch itself, as viscosity is sensitive also to the way of preparation of the solution [15]

The course of retrogradation of the marsh mallow starch and reference potato starch is presented in Fig. 1. It is evident that the rate of retrogradation of both starches is practically equal, which points to the same behaviour of the substrates in solution.

The course of degradation catalyzed by α-, β-, and γ-amylases is illustrated in Fig. 2. The marsh mallow starch is a good substrate for the amylases. Under constant conditions amylolyses proceeded with all enzyme preparations, to a certain degree, more rapidly than in case of the potato starch. The course of hydrolysis differed only with β-amylase where the originally higher rate decreased in later stages more rapidly.

Enzyme degradation was utilized in determination of purity of the isolated and physicochemically and chemically characterized starch preparation. Total hydrolysis catalyzed by combined effects

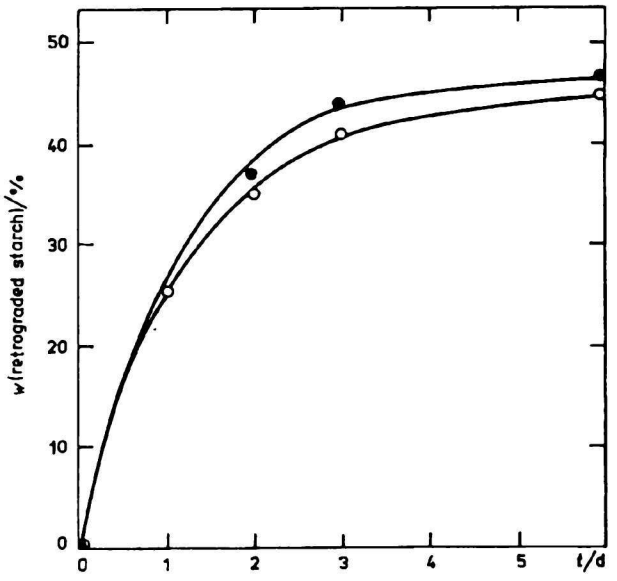


Fig. 1. Time-course of retrogradation of starches. ● Potato starch, ○ marsh mallow starch.

of α- and γ-amylases showed that the purity, expressed relative to the commercial potato starch, was 98.11 %.

The starch present in the roots of marsh mallow is similar to potato starch in composition, enzyme degradability, formation of solutions, and retrogradation and does not show any noticeable differences. After extraction of the mucilage, starch may, with certain difficulties, be isolated in the form of granules, however, removal of other residues from the damaged plant material and obtaining the starch in pure state is rather difficult.

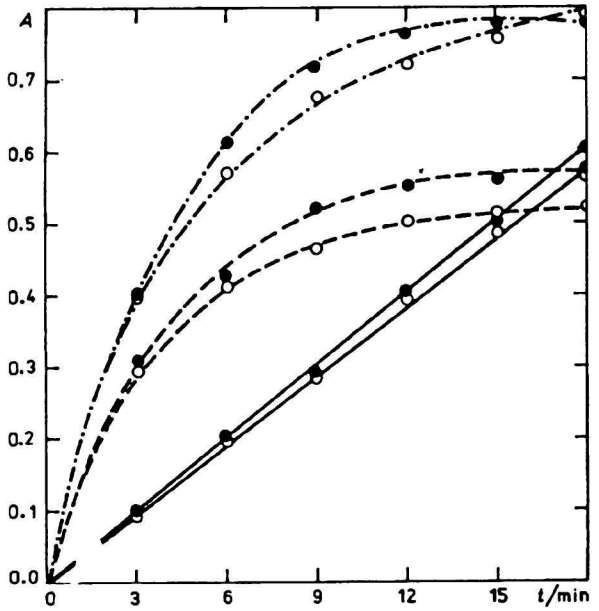


Fig. 2. Degradation of marsh mallow and potato starches by α-, β-, and γ-amylases. ● Marsh mallow starch, ○ potato starch, --- α-amylase, --- β-amylase, — γ-amylase.

Beside the starch isolated from the insoluble residue discussed above, we paid attention also to the material which was extracted together with the mucilage, but precipitated during evaporation and dialysis of the extract. We found that it was a mixture of the smallest pasty starch granules and other compounds, including colour materials. Separation of starch from this mixture is not effective.

All solid residues of the studied medicinal plant represent a starch-containing material which deserves adequate utilization, *e.g.* in fermentation industry or in other suitable microbial processing.

The present investigation completed the knowledge on α-D-glucans occurring in the roots of marsh mallow by confirming the presence of reserve starch beside the moderately branched (1→6)-α-D-glucan resembling microbial dextrans [4].

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Preparation, Characterization, and Antimicrobial Activity of 3-Alkyl-5-decyloxymethyloxazolidines

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3-Alkyl-5-decyloxymethyloxazolidines were prepared by cyclocondensation of 1-alkylamino-3-decyloxy-2-propanols with formaldehyde. The structure of the synthesized compounds was proved by IR and mass spectral data. Antimicrobial efficiency of the prepared oxazolidines was also determined.

Derivatives of oxazolidines exhibiting bactericidal, fungicidal and virucidal activity were published mainly in the years 1969—1981. The most significant groups of the active derivatives are briefly summarized in the paper [1]. The earlier publications about oxazolidines and their properties are summarized in the work by *Bergmann* [2]. In the eightieth oxazolidines were used in the larger extent as herbicides [3—7] and herbicidal antidotes [8—11].

In the human medicine, oxazolidine derivatives are applied as β-adrenergic inhibitors [12—14]. However, this effect is probably due to alkanolamines formed from oxazolidines by hydrolysis in the organism.

In our previous papers [1, 15—17] we have studied oxazolidines with alkyl chain in the position 3. This paper deals with a new type of oxazolidine derivative having long alkoxymethyl chain in the position 5. For their synthesis, we started from 1-alkylamino-3-decyloxy-2-propanols *I* which afforded required oxazolidines (*II*) by cyclization reaction with formaldehyde in the alkaline medium (Scheme 1). Ethanol and water were used as a solvent.

Alkanolamines *I* were prepared by the reaction of decyloxymethyloxirane with short-chain