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ISOLATION OF CHITIN FROM CRUSTACEANS OF THE ARAL SEA AND SYNTHESIS OF DERIVATIVES OF IT

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The isolation of chitin and the synthesis of chitosan and of the Na salt of carboxymethylchitosan from a testaceous crustacean of the Aral Sea — the shrimp Palaemon elegans *— have been carried out for the first time.*

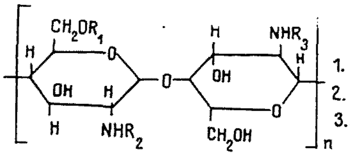
The uniqueness of the object selected for investigation consists in the fact that the shrimp species *Palaemon elegans* was introduced into the Aral Sea comparatively recently. These shrimps have now changed shape and decreased in size (from 6 to 2.5 cm). This indirectly shows mutations that have come about in connection with the unfavorable ecological situation in the Aral region.

Because of this, it may be expected that the chitin and chitin derivatives isolated will differ substantially from those obtained from other sources [1-5]. Their analysis may lead to the finding of special properties and promising new fields of application.

We are the first to have isolated chitin from the shrimp *Palaemon elegans* by alkaline hydrolysis.

Deproteination and decalcification under mild conditions enabled us to achieve a 30% yield of the desired product, which corresponds to the maximum amount of chitin in testaceous marine crustaceans. We used 3-5% NaOH, a temperature of 353-373K, and a reaction time of 150-210 min.

The chitin was deacetylated by treatment with 50% NaOH solution, giving about 45% conversion to the desired product. Chitosan is the product of the partial hydrolytic cleavage of the acetyl groups of chitin (1) and their conversion into amino groups (2), chitin being an amino compound in which the amino groups are acetylated.



1. $R_1=H, R_2=R_3=COCH_3$
2. $R_1=R_2=R_3=H$
3. $R_1=CH_2COONa, R_2=COCH_3, R_3=H$

Completely acetylated and deacetylated chitins are not found in nature and are practially impossible to obtain synthetically. The degree of acetylation calculated from the results of elementary analysis amounted to 44%.

By treating the chitosan obtained with monochloroacetic acid in isopropanol, the water-soluble Na salt of CMCh (3) was obtained with a yield of 75%.

The structures and composition of the products obtained were characterized by elementary analysis and IR spectroscopy, and their solubilities were investigated.

In the IR spectrum of the chitin, amide bands appeared clearly at 1665, 1555, and 1313 cm^{-1}, which can be assigned respectively to the absorption of carbonyl (C=O) groups, NH deformation vibrations in the plane of —CONH group, and CN bonds.

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When the chitin was deacetylated, the intensity of the absorption bands of carbonyl groups (1625 cm^{-1}) and of amide groups (3265 and 3100 cm^{-1}) fell and the intensity of a band at 3445 cm^{-1} rose, which showed the appearance of NH_2 groups. The practical disappearance of the band at 1555 cm^{-1} showed the conversion of the chitin into chitosan.

In the synthesis of the Na salt of carboxymethylchitosan (CMCh) the IR spectrum showed shifts of the absorption band for O=C-NHR from 1650 to 1670 cm^{-1} and for $-NH_2$ from 1570 to 1590 cm^{-1}, and also a decrease in the intensity of the peak in the 1313 cm^{-1} region and the appearance of a band at 1410 cm^{-1}, which may indicate the partial transformation of the chitosan into a Na salt.

The chitosan that has been synthesized has been used as a coating for cotton seeds, and trial experiments showed an intrinsic biological activity, which was expressed in an improvement in the sowing properties of the seeds (by 10-12%) and in resistance, as compared with a control (seeds not treated in any way), to the causative agent of bacterial blight in cotton (twofold fall in infestation). This agrees with information in the literature on the possession of bactericidal properties by chitosan from various sources [6].

EXPERIMENTAL

General Observations. The chitin was extracted from *Palaemon elegans* shrimps (1989) procured from the littoral zone of the Aral Sea at a depth of 20 m.

IR spectra were taken on a Specord IR-75 spectrophotometer of specimens in the form of tablets with KBr in the absorption spectral range of 400-4000 cm^{-1}.

Isolation of the Chitin. The *Palaemon elegans* shrimps were washed, dried, and ground in a KM-1 ball mill to a particle size of 1-2 mm. The pinkish brown shrimp powder was placed in a flask with a reflux condenser and was boiled in a large amount of water for 4 h. The residue was filtered off with suction and was treated at room temperature with 5% HCl (GOST 3118−77 h, proportion of HCl by weight 35%). The mixture was filtered with suction and the residue was washed with water and treated with 3% NaOH solution at 353K for 2.5 h. The alkaline solution was removed from the solid residue with suction, and the latter was washed with water. The chitin so obtained consisted of a light friable mass with a faint pink color. To eliminate pigments, the chitin was bleached with with dilute $KMnO_4$ solution and was then washed with $NaHSO_3$. Its ash content was 3.95%.

Preparation of Chitosan. The chitin was first washed with 40% NaOH solution to improve the penetration of the alkali into its pores and was then deacetylated by the action of 50% NaOH solution at a bath ratio of 1:20 and a temperature of 413K for 2 h with vigorous stirring. The product was washed free from alkali and was then washed with ethanol and dried. The chitosan so obtained consisted of a friable amorphous yellowish-grey mass soluble in 2.0% CH_3COOH and HCl and swelling in water.

Synthesis of the Na Salt of CMCh. Chitosan was first broken down with dimethyl sulfoxide at a ratio of 1:5 for 24 h to a pulpy consistency. Then the excess of solvent was eliminated, and the residue was washed with ethanol and dried. Before addition to 65% NaOH the chitosan was ground to a powder, and it was then stirred with the alkali for 1 h. The mixture was filtered and was then stirred with propan-2-ol containing monochloroacetic acid for 1 h. The resulting product was filtered again, transferred into water and neutralized, and the resulting viscous solution was precipitated with acetone in a ratio of 1:1. The NaCMCh was washed with ethanol and dried. The yield was 75% on the initial chitosan.

Treatment of Cotton Seeds. The seeds (100 g) were treated with 3 ml of a 1% solution of chitosan in 2% CH_3COOH. The trials were conducted in vegetation dishes in quadruplicate, the soil being infected with *Rizoctoni solani* grown on sterilized oats and with a seven-day culture of the causative agent of blight. Untreated seeds were used as control.

Thus, chitin has been isolated from the Aral Sea shrimp *Palaemon elegans* for the first time, and chitosan and the Na salt of carboxymethylchitosan have been synthesized from it.

REFERENCES

1. R. A. A. Muzzarelli, Chitin, Pergamon Press, Oxford (1977).
2. K. D. Vorlop and J. Klei, Biotechnol. Lett., **3**, No. 1, 9 (1981).
3. D. Knorr, Food Technol., **2**, No. 5, 183 (1984).

4. S. Toshihiro, J. Appl. Polym. Sci., **37**, No. 10, 3011 (1989).
5. G. A. Torres and M. Bi, Biochim. Organismes Mar., **16**, No. 5, 375 (1990).
6. Japanese Patent 7,319,213 (1973); Chem. Abstr., **80**, 72291 (1984).