

STUDY OF SULPHOMUCOPOLYSACCHARIDES OUT OF *Mya arenaria* L.Molnar I. A.,¹ Drimbărean E.¹ and Mirza M.²¹Institute of Chemical Pharmaceutical Researches - București²Romanian Marine Research Institute - Constanța

ABSTRACT:

The content, chemical and pharmaceutical properties of the sulphomucopolysaccharides (MPS) from *Mya arenaria* L. have been studied. Out of 1 kg dry tissue 19.2 g of partially purified mucopolysaccharides were obtained, having 5.67 % N, 1.42 % S, 2.7 % hexuronic acid, 9.2 % humidity and 4.8 % residium on calcination.

The tissues of the vertebrates, besides heparine, with a very intense activity in anticoagulation, also contains another group of acid sulphomucopolysaccharides, whose hypocholesterolemiant and hypolipemiant activity is similar to that of the heparine, but possesses a much lesser anticoagulant activity than the heparine. The aim of our paper is the identification of the existence of these substances in *Mya arenaria* L., the species who lives in Black Sea, the isolation and the determination of some physical-chemical and pharmaceutical properties. The existence of MPZ in mollusk was very little researched. The works in this pro-

blem were effected by: DOYLE (3), WADO KOJI and al. (10; 11), CIFONELLI and MATHEWS (2). The pharmacological properties of these MPS have not been researched.

MOLNAR and al. (8) were obtained from dry tissue of Mytilus galloprovincialis, the sulphomucopolysaccharides acides and were studied on them some physical-chemical properties and from a pharmaceutical point of view, the hypolipemiante and hypocholesterolemiante action on the rat intoxicated with Triton A-20. The physical-chemical and pharmaceutical properties of the obtained sulphomucopolysaccharides from Mytilus have a greater semblance with those obtained by BIANCHINI (1) and MOLNAR and al. (4; 5; 7), from the cattle and pork duoden and from the fowl intestine. In the present paper, we have studied the mucopolysaccharides of Mya arenaria, as a continuation of previous works.

MATERIAL AND METHODS

In our experiments we have studied Mya arenaria gathered on the 4-th of August 1973 at the Cape of Mydia (north Constantza). The mussels were of different size, as they are found naturally. The tissue was separated from the shells and used totally.

The preparatory and analitical works were described in a previous work, MOLNAR and al. (8).

RESULTS AND DISCUSSIONS

The dry tissue contains 8,39 pc. total lipides. Achieving the extraction of the acid mucopolysaccharide by the method described, out of 1 kg dry degreased tissue, 19,2 g raw preparation. The preparation obtained has not the degree of purity corresponding to that obtained by Bianchini but it is similar to those obtained by MOLNAR and al., out of various tissue of the digestive tube from cattle, hog, fowls and Mytilus galloprovincialis and is qualitatively good for establishing certain physical-chemical and pharmaceutical properties. The following analytical data have been obtained for the our preparations (Table 1).

The identification of mucopolysaccharide achieved according to the technique described in a previous work, shows the maximum of extinction at 590 nm.

Table 1

Analytical results obtained with MPS

Loss on drying at 105°C %	9.2
Residium on calcination %	4.8
Total N ₂ %	5.67
Total S %	1.42
Hexuronic acid %	2.7
Anticoagulant activity corresponding to IU Heparine/mg	0.65
Electroforetic migration speed U	5.0 - 5.4
Metachromatic reaction on toluidine-blue paper	intensely violet
Maximum of extinction λ = nm	590

In comparison with a mucopolysaccharide sample prepared by us from Mytilus, a surprising similarity is seen between the absorption spectres of mucopolysaccharide combination with toluidine-blue out of these two mollusk species (Fig.1). In a previous work, the authors shows that the absorption spectres for the methacromatic reaction of mucopolysaccharides prepares by us from pork pancreas and Mytilus galloprovincialis have a great similarity, which proves that the structures of these mucopolysaccharides coming from totally different species are very much related. Because the obtained spectres in this reaction with MPS out of Mytilus galloprovincialis and Mya arenaria have a great semblance means that their structure is very much related.

On studing the methacromatic reaction with toluidine-blue in the function of the MPS content in the sample for the purified sample, there has been visually stated the appearance of a violet shade whose intensity increases with the MPS concentration in the sample, reaching a maximum, after which a second reaction appears and the colours of the samples pass gradually to light blue. This colour modification was quantitatively researched spectrophotometrically between 500 - 700 m. In the first stage of the reaction the moving of the maximum of extinction of the toluidine-blue to shorter wave-lengths is noticed, this being known under the name of metachromatic reaction; in the second stage the

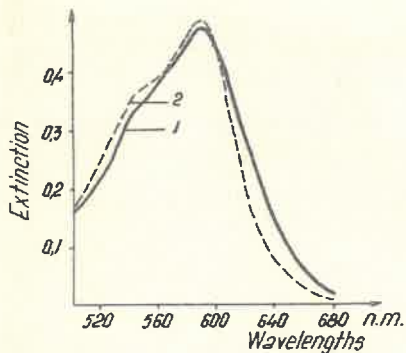


Fig. 1
Reaction for the identification of the mucopolysaccharides
1. MPS from Mya arenaria.
2. MPS from Mytilus galloprovincialis.

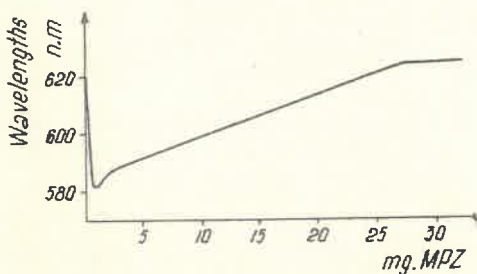


Fig. 2
The modification of the position of the maximum of the extinction depending on the MPS concentration in the 15 ml sample.

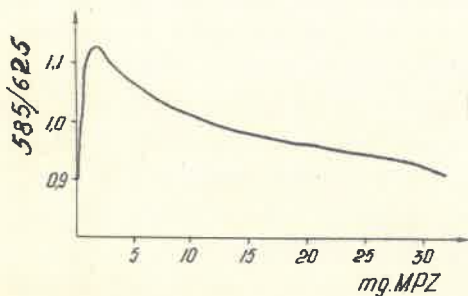


Fig. 3
The modification of the extinction ratio at 585/625 nm on depending on the MPS quantity in the sample.

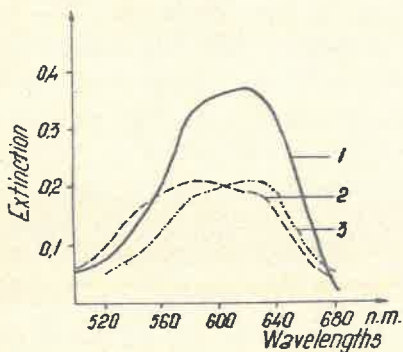


Fig. 4
The absorption spectre of the toluidine-blue in the presence of the mucopolysaccharide from Mya arenaria, in various quantities.
1. Toluidine-blue solution.
2. Toluidine-blue and 2 mg MPS in the sample.
3. Toluidine-blue and 32 mg MPS in the sample.

maximum of extinction moves to longer wavelengths. At a concentration of 0,8 mg MPS in the 15 ml sample, maximum of extinction is at 582 nm, and on concentrations stronger than 24-32 mg MPS in the sample, at 625 nm, slightly moved to longer wave lengths in comparison with 620 nm, the maximum of extinction of the toluidine-blue (Fig.2).

Following the extinction modifications at 620 nm, the wave lengths corresponding to the maximum of extinction of the toluidine-blue in the first stage of the reaction, a decrease of the value of the extinction is stated, while in the second stage an increase is seen. The reaction can be best traced by the modifications of the extinction values ratios for the two wave lengths correspondingly chosen function of the MPS content in the sample. The extinction ratios 585 and 625 nm wavelengths are shown in Fig.3. Here, in the first stage an increase, and in the second a decrease of the ratio is noticed. The maximum value was noticed at a content on 2,2 mg MPS in the sample. The spectres of the combinations given by MPS in various quantities in samples with the toluidine-blue in comparison with the toluidine-blue are shown in Fig.4.

Comparing the absorbtion spectres, obtained in the first stage of the reaction, the so called metachromatic reaction, for MPS obtained out of Mya arenaria with that of Mytilus galloprovincialis previous obtained by MOINAR and al., it can be seen that they have a common characteristic, that is the maximum position of extinction at approached wavelengths (582 respectively 580 nm), but in the second stage of the reaction, the maximum of extinction is at 625 nm for both preparation.

The result of the pharmaceutical control of the preparat are to be seen in the table below.

Table 2

The antilipemic effect
A. Optic density

Lot	Number of animals	D.O.	P	Protective x) effect %
Control	20	0.08±0.045	-	-
Intoxicated with triton	20	1.05±0.009	-	-
Intoxicated and treated with MPS	20	0.764±0.036	0.05	28.3(25.19-32.87)

Table 2 (continuation)

B. Total lipidemy

Lot	No. of animals	Total lipides mg in 100 ml	P	Protective effect %
Control	20	55.5 ± 5.18	-	-
Intoxicated with triton	20	344.0 ± 5.20	-	-
Intoxicated and treated with MPS	20	302.2 ± 8.76	0.01	14.5(13.66-15.40)

Table 3

The anticholesterolemic effect

Lot	No. of animals	Cholesterol in mg in 100 ml	P	Protective effect %
Control	20	38.10 ± 2.60	-	-
Intoxicated with triton	20	131.95 ± 8.60	-	-
Intoxicated and treated with MPS	20	112.50 ± 5.35	0.2	20.7(17.68-25.57)

x) Fiducinal limits to a protective effect for $P=0.05$ in brackets.

xx) Fiducinal limits to a protective effect calculated by method of SIMIONOVICI and al.(9) in brackets.

From these results one can see that MPS of Mya present a protective action with the hyperlipidemy and the hypercholesterolemey of the Triton -20 intoxicated rats. This effect is similar, but poorer than MPS of Mytilus galloprovincialis.

The physical-chemical and pharmaceutical characteristics studied, show a great similarity between the mucopolysaccharides extracted from Mya arenaria and those from pork, cattle, fowls.

CONCLUSIONS

The tissue of Mya arenaria contains a sulphomucopolysaccharide which has physical-chemical properties very close to those from the cattle and pork duodenum, their intestine, stomach and pancreas, and from the fowl intestine and Mytilus galloprovincialis, such as: electrophoretic migration speed, teaction of colouring with toluidine-blue.

From the view point of the pharmaceutical activity, the mucopolysaccharide from Mya arenaria L., presents a protective

activity against hiperlipidemy and hypercholesterolemy, experimented on the rat intoxicated with Triton A -20, but the anticoagulant effect is insignificant, almost negligible, by which they are different from the heparine. These pharmacological properties are common in MPS aut of tube digestive tissue from mammals, fowls and from mollusk *Mytilus galloprovincialis*, previous studied, thus these MPS, are from the same category of substances.

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