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METABOLITES FROM SPONGES AS INHIBITORS OF β-1,3-GLUCANASE

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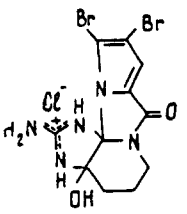
The action of a number of aromatic and nitrogen heterocyclic metabolites from sponges on β-1,3-glucanase Lo from the bivalve mollusk Chlamys albidus has been investigated. The greatest inhibiting action on the activity of α-1,3-glucanase Lo was shown by puupehenone derivatives.

The increasing interest in enzyme inhibitors is connected with their practical use in medicine for the diagnosis and treatment of a number of diseases [1, 2]. Specific inhibitors are used for studying the mechanism of the action of enzymes [3].

We have investigated the action of a number of aromatic and nitrogen heterocyclic metabolites from sponges on β-1,3-glucanase Lo from the bivalve mollusk Chlamys albidus living in the Sea of Japan.

Inhibiting action was determined from the capacity of solutions of the metabolites for interfering with the interaction of Lo with laminarin. The dependence of the inhibiting action on the concentration of the substance was determined. Table 1 gives the amounts of substance in a sample causing 50% inhibition of $2 \cdot 10^{-2}$ activity units of the enzyme.



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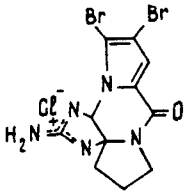

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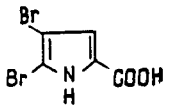

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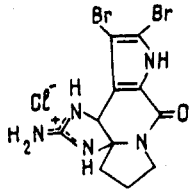

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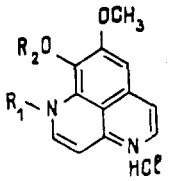
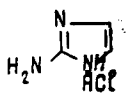


5. $R_1 = H$, $R_2 = CH_3$

6. $R_1 = CH_3$, $R_2 = H$



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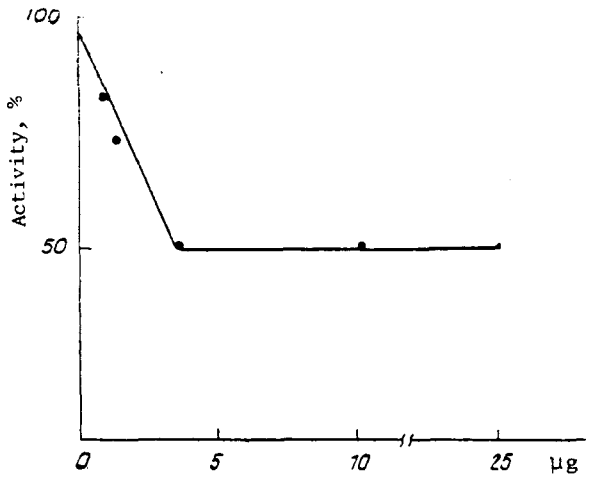
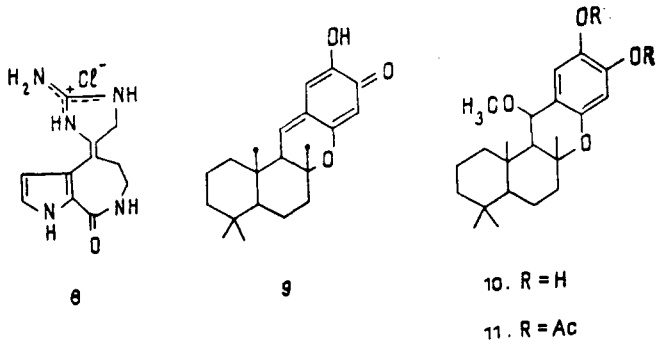


Fig. 1. Inhibition of β-1,3-glucanase by puupehenone (9).



The results of the experiments showed that with respect to their effect on the activity of β-1,3-glucanase Lo the substances investigated can be divided arbitrarily into three groups:

1. Polycyclic bromopyrrole compounds (2, 3, 4, 8) up to a concentration of 50 μg in the sample did not change the activity of the enzyme, i.e., were practically not inhibitors of β-1,3-glucanase;

2. Substances 1, 5, and 7 caused 50% inhibition of the β-1,3-glucanase in fairly high concentration – 50-60 μg in the sample. However, the prospect of the use of these compounds is not excluded. In some cases, the inhibiting effect can be enhanced by changing functional groups. Thus, the inhibiting capacity of compound 5, which is an aaptamine derivative is three times greater than that of aaptamine (compound 6) itself;

3. The third group of substances (9, 10, 11), which consists of puupehenone and its derivatives, lowers the activity of the enzyme by 50% in concentrations of from 4 to 25 μg in a sample. It has been established that puupehenone possesses a high antimicrobial activity [4]. It is assumed that this activity is due to the fragment including an α,β-unsaturated ketone with an OH group in the α-position, since on passing to compounds 10 and 11 the antimicrobial activity decreased. A similar relationship was observed in the change of inhibiting capacity of these substances, although these changes were less pronounced. The inhibiting effect of puupehenone derivatives is probably largely due to the binding of the hydrophobic part of the molecules of the given substances with the surface of the protein globule of β-1,3-glucanase.

Some peculiarity of the action of puupehenone on β-1,3-glucanase was detected. Analysis of the inhibition curve (Fig. 1) showed that the loss of 50% of the activity of the enzyme set in at the minimum concentration of the substance in the sample of 4 μg. A further increase in the concentration did not lead to a change in the activity of the β-1,3-glucanase. In this case, a reversible nature of the inhibition is not excluded. Reversible inhibitors are of great value for studying the mechanism of the action of enzymes.

TABLE 1. Action of Metabolites from Fungi on β-1,3-Glucanase Lo

Compound	Solvent	Amount of substance in the sample causing 50% inhibition. μg
1. Dibromoagelaspongin hydrochloride [8]	H_2O	50
2. Dibromophakellin hydrochloride [9]	H_2O	50, does not inhibit
3. 4,5-Dibromopyrrolecarboxylic acid [9]	H_2O	50, does not inhibit
4. Dibromoisophakellin hydrochloride [10]	H_2O	50, does not inhibit
5. Aaptamine hydrochloride [11]	H_2O	60
6. 1-N-Methyldemethylaaptamine hydrochloride [11]	H_2O	20
7. α-Aminoimidazole hydrochloride [12]	H_2O	65
8. Debromohymenialdisine hydrochloride [13]	H_2O	50, does not inhibit
9. Puupehenone [4, 14]	DMSO	4
10. 11-Methoxypuupehenone [14]	DMSO	17
11. 11-Methoxypuupehenone diacetate [14]	DMSO	25

EXPERIMENTAL

The metabolites investigated were isolated from tropical sponges. Information on the determination or identification of their structures is given in the references cited in Table 1. The endo-β-1,3-glucanase Lo was isolated from the bivalve mollusk Chlamys albidus by the method described in [5]. Laminarin was obtained from the brown alga Laminaria cichorioides [6].

Inhibition Procedure. To 100 μl of the enzyme in 0.05 M succinate buffer containing 0.1 M NaCl was added 50 μl of a solution of one of the substances under investigation in dimethyl sulfoxide (DMSO). The mixture was kept at 25°C for 15 min and then 400 μl of laminarin solution (1 mg/ml) was added and incubation was carried out at the same temperature for 15 min. The residual activity was determined from the increase in the amount of reducing sugars found by Nelson's method [7]. The influence of amount of DMSO introduced into the reaction mixtures was taken into account in a control experiment. As the unit of activity we took the amount of enzyme producing 1 μmole of glucose/min.

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