By using eluents consiting of methanol–water–acetic acid (70:30:0.005, 70:30:0.003, and 70:30:0.002; pH from 5.13 to 5.60) we succeeded in separating a model mixture including all three alkaloids: deoxypeganine, peganol, and deoxyvasicinone in a ratio of 1:2:1 (Fig. 1).

The most effective separation of the alkaloids under analysis was observed in a variant of ion-pair chromatography; the main factor affecting the separation of the peaks on chromatography was the pH of the medium.

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A NEW ANTIBIOTIC FROM A MARINE STREPTOMYCETE

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In recent years with the pursuance of chemical and biochemical investigations on marine macrobionts, the number of publications on the study of the metabolites of marine symbionts and free-living microorganisms (actinomycetes, bacteria, fungi, microalgae) has risen [1, 2]. Interest in such investigations is due to the possibility that they open up a biotechnological method of obtaining compounds of practical importance.

During the ninth expeditionary cruise of the Scientific Research Ship Akademik Oparin, from a sample of sea bottom (Palmyra Island, 5°52'72" N, 162°8'03", W, May 10, 1989; Line Archipelago, USA) we isolated an antibiotic-producing actinomycete belonging to the genus Streptomyces (strain KMM 9BS12A).

Cultivation of the strain in liquid media with sea water led to the accumulation of antibiotic compounds. After the separation of the mycelium, the culture liquid was filtered through a KhM-50 filter in an Amicon-8050 ultrafiltration cell. The antibiotic components, which had remained in the cell, were chromatographed twice on a column of Sephacryl S-200 (Pharmacia). The biological testing of the fractions was carried out with the use of a test culture of the microorganism Staphylococcus aureus. The active eluates were combined and lyophilized.

Subsequent chromatography on Servacel TEAE-23 (Reanal) led to the isolation of an antibiotic which we have called palmyromycin. Its homogeneity was determined on electrophoretic analysis (polyacrylamide gradient gel 4/30, Pharmacia, Tris buffer pH 8.4). This compound possessed no cytostatic action in relation to tumor cells of Ehrlich's carcinoma and showed no proteolytic activity. It inhibited the growth of the Gram-positive bacteria St. aureus and Bacillus subtilis.

The molecular mass of palmyromycin was calculated from the results of electrophoresis under the conditions given above with the following standard proteins: bovine albumin – 67 kDa; lactate dehydrogenase – 140 kDa; and catalase – 232 kDa (Serva). It proved to be ~85 kDa. These results correlated well with the behavior of the substance in gel filtration of Sephacryl S-200.

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Treatment of palmyromycin with 2-mercaptoethanol followed by electrophoresis under denaturing conditions (gradient polyacrylamide gel 9/25, SDS buffer, pH 6.8) led to the detection of only one spot, with a mass of approximately 18 kDa. It may be assumed that the antibiotic probably consists of five identical subunits.

Thus, we have shown that palmyromycin is a high-molecular-mass protein possessing antibiotic properties. No proteins of similar mass possessing antibacterial action have previously been isolated from actinomycetes.

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