SEPARATION OF THE ALKALOIDS DEOXYPEGANINE, PEGANOL, AND DEOXYVASICINONE BY THE HPLC METHOD

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The literature on the HPLC analysis of quinazoline alkaloids is sparse and is mainly devoted to the separation of peganine and vasicinone, of deoxypeganine and deoxyvasicinone, and of tetramethylenequinazolone and deoxyvasicinone [1-3].

We have separated the alkaloids deoxypeganine, peganol, and deoxyvasicinone by microcolumn, high-performance liquid chromatography with the aim of a further study of their interconversions. Chromatographic analysis was conducted on a Milikhrom 1A instrument with the UV detection of the substances being separated, at wavelengths of 254, 278, and 296 nm. A Silasorb-C_{18}, 2 × 80 mm, column was used, with particles having a size of 5 μm (Czechoslovakia). As markers we took the alkaloids deoxypeganine (I), deoxyvasicinone (II), and peganol (III) isolated from the epigeal part of Peganum harmala [4]; and also deoxyvasicinone obtained by the condensation pf anthranilic acid with butyrolactam, deoxypeganine obtained by the reduction of deoxyvasicinone [5], and peganol synthesized from deoxypeganine [6]. The solvents used as eluents were binary mixtures of methanol and water. The optimum ratios of the components of the mixture, selected experimentally, were 70:30 and 60:40. To increase the efficiency of chromatographic separation and to achieve a variant of ion-pair chromatography, compounds of acidic nature (perchloric and acetic acids, phosphate buffer) were added to the eluent [1, 2].

In the analysis of a mixture of peganol and deoxyvasicinone good separation took place even in strongly acid media, i.e., in methanol–water–perchloric acid (70:30:0.01 and 60:40:0.01) systems having pH values of from 2.50 to 2.63. Such a composition of the eluent also proved to be suitable for the separation of a binary mixture of deoxypeganine and deoxyvasicinone. The choice of the conditions of separation of a mixture of deoxypeganine and peganol was a fairly complex task. It was natural to assume that some definite interval of pH values exists within which selectivity exists in the protonation of the nitrogen atoms of deoxypeganine and peganol. In strongly acid media there was only an insignificant difference in the retention times of these bases, peganol being eluted from the column somewhat earlier than deoxypeganine. A good separation of this mixture was achieved in a flow of eluent with pH 5.13 and above, i.e., in the methanol–water–acetic acid (70:30:0.005; pH 5.13) and methanol–phosphate buffer (50:50, pH 5.96, and 40:60, pH 6.74) systems. In a weakly acid medium, the order of elution of the components under consideration changed – the deoxypeganine was eluted first. It must be mentioned that we gave preference to a system including acetic acid in view of its greater eluting capacity.

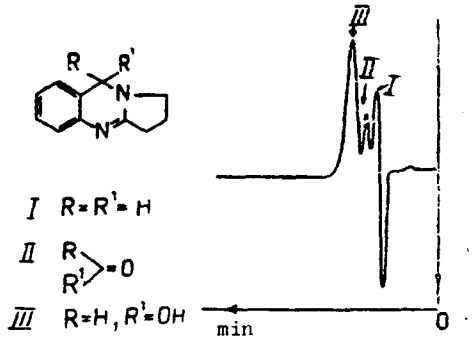

Fig. 1. Separation of a mixture of the alkaloids deoxypeganine, peganol, and deoxyvasicinone. Silasorb C_{18}, 2 × 80 mm, 5 μm, column., Eluent: methanol–water–acetic acid (70:30:0.005); rate of elution: 0.2 ml/min; detector: UV, 296 nm.

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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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