where $[S_0]$ = 17.9 mg/ml is the concentration of hemoglobin; [I] = 2.23 mg/ml is the concentration of gramicidin S; and $K_{M(app)}$ = 5.7 mg/ml is the Michaelis constant for the hydrolysis of hemoglobin by equine pepsin (it was determined in the same way as for porcine pepsin, see Fig. 1, line a); and a is the ratio of the activities of pepsin in the absence and in the presence of gramicidin S, which under the given conditions is 1.21. Calculation gave $K_I$ = 2.6 mg/ml (or 2.0 mM).

## SUMMARY

1. It has been shown that polypeptide antibiotics — bacitracin and gramicidin S — inhibit carboxylic proteinases — porcine and equine pepsins. Bacitracin inhibits pepsin with $K_I$ = 2.3 mM, and gramicidin S with $K_I \sim$ 2 mM.

2. The results obtained permit a theoretical foundation for the use of bacitracin and gramicidin S and also of other polypeptide antibiotics as ligands for the biospecific chromatography of proteinases.

## LITERATURE CITED

1. R. E. Galardy, M. P. Printz, and L. C. Craig, Biochemistry, 10, 2429 (1971).
2. K. K. Mäkinen, Int. J. Protein Research, 4, 21 (1972).
3. M. L. Anson, J. Gen. Physiol., 22, 79 (1938).
4. W. T. Jackson, M. Schlamowitz, and A. Shaw, Biochemistry, 5, 4105 (1966).
5. B. M. Stepanov, G. I. Lavrenova, K. Adli, M. V. Gonchar, G. N. Balandina, M. M. Slavinskaya, and A. Ya. Strongin, Biokhimiya, 41, 294 (1976).
6. V. M. Stepanov, G. I. Lavrenova, G. N. Rudenskaya, M. V. Gonchar, L. S. Lobareva, E. K. Kotlova, A. Ya. Strongin, L. A. Baratova, and L. P. Belyanova, Biokhimiya, 41, 1285 (1976).
7. V. M. Stepanov, L. S. Lobareva, G. N. Rudenskaya, V. P. Borovikova, G. G. Kovaleva, and G. I. Lavrenova, Bioorg. Khim.. 3. 831 (1977).
8. M. M. Chernaya, K. Adli, G. I. Lavrenova, and V. M. Stepanov, Biokhimiya, 41, 732 (1976).
9. T. I. Vaganova, L. V. Lastovetskaya, A. Ya. Strongin, L. A. Lyublinskaya, and V. M. Stepanov, Biokhimiya, 41, 2229 (1976).
10. L. F. Matyash, T. L. Voyushina, S. V. Belyaev, and V. M. Stepanov, Prikl. Biokhim. Mikrobiol., 11, 604 (1975).

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# STUDY AND ISOLATION OF VANADYLPORPHYRINS FROM THE PETROLEUM OILS OF THE BUZACHI PENINSULA

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The porphyrin fragments of petroleum oils discovered by A. Treibs in 1934 [1] have long attracted the attention of research workers. All porphyrin pigments are related compounds of the porphins. A fairly large number of porphyrins differing from one another by their side-chain substituents is known: the majority of them have been obtained synthetically, and a considerable number have been isolated from biological sources [2].

Several types of porphyrins have been detected in petroleum oils and ancient sediments [3, 4]. Porphyrins may be present in petroleum oils both in the free form and in the form of complexes with heavy-metal ions as a vanadium complex [5], an iron complex [6], and a nickel complex [7, 8]. The complexes of other metals with porphyrin have not yet been identified with certainty.

There is no information in the literature on the isolation and structure of the porphyrin fragments of the petroleum oils of Kazakhstan. We have limited ourselves to determining the vanadylporphyrins in the petroleum oils of the Buzachi peninsula and to isolating them.

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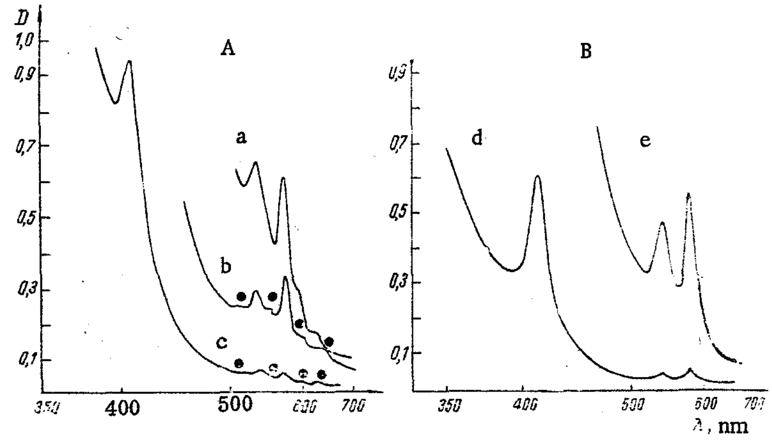

Fig. 1. Absorption spectra of vanadylporphyrins in the visible region: a) isolated by dioxane from Karazhanbas petroleum; b, c, and •) maxima of the absorption bands of free porphyrins; d and e) typical absorption spectra of vanadylporphyrins after chromatographic purification.

The spectra of all the petroleum oils investigated show a strong absorption line due to free radicals and absorption lines due to $V^{4+}$ in the form of vanadium compounds of porphyrin. The electron spin resonance (ESR) absorption spectrum of $V^{4+}$ consists of two hyperfine structures: with a splitting constant $A_{II}$ for the $g_{II}$ lines and with a constant $A_{I}$ for the $g_{I}$ lines. The determination of the parameters of the spin Hamiltonian from the anisotropic hfs of the spectra of the petroleum oils investigated gave $A_{II} = 0.0163$ cm$^{-1}$, $A_{I} = 0.0060$ cm$^{-1}$, which agrees well with the constants of the spin Hamiltonian of complexes of $V^{4+}$ with porphyrin [9].

The porphyrin fragments of the petroleum oils were also studied spectrophotometrically. They were extracted from the crude oils by polar solvents immiscible with petroleum.

Fig. 1A shows the visible spectrum of the porphyrin fragment isolated by dioxane from Karazhanbas petroleum (1:1); the configuration of the peaks and their positions in the spectrum (411, 534, and 573 nm) are characteristic for vanadium porphyrins [7]. To obtain more characteristic absorption spectra of vanadylporphyrins in the visible region of the spectrum, the residue of the extract was deposited on a column of alumina. Using a known procedure, the sparingly polar compounds were eluted with hexane and cyclohexane. The porphyrin complexes of vanadium were eluted with dioxane. Typical absorption spectra of the vanadylporphyrins are given in Fig. 1B. The same type of spectrum is observed for Kalamkas and Severo-Buzachinsk oils.

On the background of the absorption spectrum of the vanadylporphyrin extracts from the Karazhanbas oil there are several bands in the visible region belonging to free porphyrins (see Fig. 1A, curve a), and therefore the Karazhanbas petroleum was also studied for porphyrins. A solution of 10 g of the oil in an excess of benzene was made, and then the benzene was evaporated off in vacuum at 70°C. The asphaltenes were precipitated by the addition of isooctane. The procedure was repeated four times. The oil free from asphaltenes was transferred to a chromatographic column. Elution was carried out as described above. The visible spectra obtained by the method used for the determination of the vanadylporphyrins showed the presence of porphyrins in the oil (Fig. 1A, curves b and c). The intensities of the lines rose, and in the visible region of the spectrum there were bands with $\lambda_{max}$ 510, 549, 573, and 633 nm.

The total amount of vanadylporphyrins in the samples of petroleum oils investigated was determined directly by the ESR method from the hyperfine structure spectrum of the complexes of tetravalent vanadium with porphyrin. The amount of vanadylporphyrins in the petroleum oils investigated was determined by the method of Roberts et al [11].

The concentration standard used was a Martyshinskaya petroleum oil with a known content of vanadylporphyrin ($0.1 \cdot 10^{-3}$ M):

| Deposit | Amount of vanadylporphyrins $10^{-3}$ M |
|---|---|
| Karazhanbas, well 105 | 4 |
| Karazhanbas, well 108 | 3.55 |
| Severo-Buzachinsk, well 175 | 2.7 |
| Kalamkas, well 3 | 2.05 |

Thus, the petroleum oils of the Buzachi peninsula are very rich in vanadylporphyrin.

We studied the selectivities of dioxane, dimethylformamide, and acetone in the isolation of the vanadylporphyrin complexes from the Karazhanbas and Kalamkas oils. In many cases the amounts of metalloporphyrins in the extracts obtained from the oils were determined spectrophotometrically [12]; however, the determination of vanadylporphyrins by this method is difficult, since when the amounts of resins and asphaltenes are considerable the absorption spectrum becomes diffuse. Consequently, a preliminary chromatographic purification of the vanadylporphyrins from the extract or from the oils is desirable. This method of determining vanadylporphyrins is lengthy and analytically difficult.

In the present paper we give the results of a determination of the vanadylporphyrins in extracts and petroleums by the ESR method. Our results show that this method is more convenient for studying petroleum oils and extracts with low contents of vanadylporphyrins (at concentrations of from $10^{-5}$ M).

A great advantage of the ESR method in comparison with the spectrophotometric method is the rapidity of its performance and the accuracy of the determination of the concentration of vanadylporphyrins.

As can be seen from the results given below, dimethylformamide and dioxane extract greater amounts of the vanadylporphyrins (% of the total amount):

| Solvent | Karazhanbas oil, well 105 | Kalamkas oil, well 3 |
|---|---|---|
| Dioxane | 37 | -- |
| Dimethylformamide | 27 | 29 |
| Acetone | 20 | 18 |

Dioxane is usually considered a weaker solvating solvent. We [13] have shown the considerable solvating capacity of dioxane in an investigation of the ESR spectrum of the anion radical of naphthalene in a mixture of dioxane and tetrahydrofuran (1:1).

In this case, the increase in the concentration of vanadylporphyrin in the extract is apparently connected with the number of molecules of dioxane adopting the boat form close to the $V^{4+}$ cation.

## EXPERIMENTAL

The samples of petroleum oils were freed from water and mechanical impurities. All the ESR measurements were performed on an RÉ-1301 radiospectrometer and a Varian E-12 spectrometer.

Identical molybdenum glass tubes which gave no appreciable ESR signal were filled with the oil and with the extract of vanadylporphyrin complexes to a height of 35 mm.

The electronic adsorption spectra of the vanadylporphyrin and the spectrum of the free porphyrin were recorded on a "Specord UV-Vis" spectrophotometer at room temperature.

## SUMMARY

1. The vanadylporphyrin complexes of the petroleum oils of the Buzachi peninsula have been studied by the ESR method and spectrophotometrically. The results of the investigation show that the ESR method is the most convenient for determining small amounts of vanadylporphyrins in petroleum oils and extracts.

2. The selectivity of various solvents in the extraction of vanadylporphyrin complexes from petroleum oils, as determined by the ESR method, is discussed.

## LITERATURE CITED

1. A. Treibs, Ann, Chem., 509, 103 (1934).
2. G. P. Gurinovich, A. N. Sevchenko, and K. N. Solov'ev, Usp. Khim., 173, (1963).
3. L. R. Fisher and H. N. Dunning, Annal. Chem., 31, 1394 (1959).
4. H. Fischer and H. Orth, Die Chemie des Pyrrols, Vol. II. Akademie Verlag, Leipzig (1937).
5. A. Treibs, Angew. Chem., 49, 682 (1936).
6. N. P. Kuznetsova, Author's Abstract of Candidate's Dissertation, Institute of Combustible Minerals, Academy of Sciences of the USSR (1946).
7. E. A. Glebovskaya and M. V. Vol'kenshtein, Zh. Obshch. Khim., 18, 1440 (1948).
8. H. H. Dunning, Ind. Eng. Chem., 45, 1759 (1953).
9. D. E. O'Reilly, J. Chem. Phys., 29, 1188 (1958).
10. W. W. Howe, Anal. Chem., 33, 1288 (1961).
11. E. M. Roberts, R. L. Rutledge, and A. P. Wehner, Anal. Chem., 33, 1879 (1961).
12. R. Nasirov and S. P. Solodovikov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 5, 1166 (1973).
13. M. F. Shostakovskii, O. V. Serebrennikova, R. F. Ivanitskaya, and V. I. Titov, Geokhim., 7, 1100 (1974).