Dependence of the Stability of the Immobilized Enzymes on the Temperature. To 1 ml of 0.1 M acetate buffer with pH 4.5 was added 10 mg of immobilized enzyme. The mixture obtained was kept at 10-70°C for 1 h. Then the buffer was decanted off at 20°C and the proteolytic activity was determined under static conditions.

Dependence of the Proteolytic Activity on the pH. To 1 ml of a 2% solution of hemoglobin with pH 1.0-6.0 (1 M HCl and 0.1 M acetate buffer) was added 10 mg of immobilized enzyme, and the proteolytic activity was determined under the static conditions.

Dependence of the Proteolytic Activity on the Temperature. To 1 ml of a 2% solution of hemoglobin with pH 1.8 was added 10 mg of immobilized enzyme, and the proteolytic activity was determined under static conditions at temperatures of 10-70°C.

The preparation of aspergillopepsin A was kindly given to us by V. I. Ostaslavskaya and E. K. Kotlova.

SUMMARY

Highly purified porcine pepsin and aspergillopepsin A immobilized on amino-Silochrome have been obtained. The enzymatic properties of these insoluble derivatives have been studied.

LITERATURE CITED

1. A. B. Ryle, Int. J. Peptide Proteins Res., 4, 123 (1972).
2. O. Valentova, J. Turkova, R. Lapka, J. Zima, and J. Coupek, Biochim. Biophys. Acta, 403, 192 (1975).
3. W. F. Line, A. Kwong, and H. H. Weetall, Biochim. Biophys. Acta., 242, 194 (1971).
4. L. S. Lobareva, G. G. Kovaleva, M. P. Shimanskaya, and V. M. Stepanov, Biokhimiya, 37, 198 (1972).
5. T. A. Solov'eva, S. V. Belyaev, and V. M. Stepanov, Khim. Prirodn. Soedin., 398 (1977).
6. V. Kh. Akparov and V. M. Stepanov, Prikl. Biokhim. Mikrobiol., 13, 141 (1977).

ISOLATION AND ANTIBIOTIC PROPERTIES OF cis-HEXADECA-4,7,10,13-TETRAENOIC ACID FROM THE ALGA *Scenedesmus obliquus* UA-2-6

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UDC 582.26:615.33+547.915:665.3

There is information on the manifestation by extracts from the cells of green [1] and blue-green [2] algae of antibiotic properties and of the therepeutic effectiveness of these preparations [3]. Sarganin and chonalgin isolated from marine forms of brown algae have proved to be more active than penicillin and nystatin [4].

The assumption exists that the antibiotic properties of preparations from the greenalgae *Chlorella* and *Scenedesmus* are due to the presence in their cells of chlorophylls [5] and fatty acids [6], but there is no information on the isolation and identification of substances possessing such activity.

The capacity of local strains of the organisms *Scenedesmus acuminatus* UA-2-7 and *Scen. obliquus* UA-2-6 for producing antibiotic substances having a lipid nature has been detected previously [7, 8]. In this communication we give information on the isolation from the biomass of *Scen. obliquus* UA-2-6 of cis-hexadeca-4,7,10,13-tetraenoic acid and its antibiotic properties.

A lipid extract was obtained from the air-dry biomass of the *Scenedesmus*. The extract consisted of a dark green oily liquid with a peculiar odor. The compositions of the classes

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of lipids of the extract were determined by thin-layer chromatography (TLC) in systems 1 and 2. Provisional identification of the classes was carried out by comparing the R_f values of the spots with literature information [9] and with the corresponding values of markers: triglycerides, free fatty acids, and β-sitosterol. The antibiotically active fractions were revealed on a parallel chromatogram in system 1 after the elimination of the solvent from it by bioautography on a background of the bacterium *Bacillus subtilis* VKM V-428.

On chromatography in system 1, the total lipids were separated into 10 spots, of which five were colored. The colored spots with R_f 0.95, 0.45, and 0.10 related to carotenoids, and those with R_f 0.15 and 0.08 to chlorophyll pigments. Among the others we identified triglycerides (R_f 0.8), free fatty acids (FFAs, R_f 0.35), and free sterols (R_f 0.17). On a bioautogram of the lipid extract from the algae two zones suppressing the growth of the test organism appeared, with R_f 0.32 and 0.05. The zone of greater area with R_f 0.32 corresponded to the lower part of the slightly extended spot of the FFAs. The second zone (R_f 0.05) corresponded to an unidentified class of lipids on the initial chromatogram. We likewise did not identify the substances of spots with R_f 0.52 and 0.0. Two-dimensional TLC in systems 1 and 2 confirmed this assignment. In the present paper we considered the antibiotically active substances giving on a bioautogram a zone with R_f 0.32.

To isolate the FFAs, the total lipids of *Scenedesmus* were fractionated on a column of silica gel. Crude fractions containing FFAs were combined and treated with sodium carbonate solution to remove traces of nonacidic impurities.

The UV spectrum of the FFAs had weak absorption at λ 232 nm ($E^{1\%}_{1\,cm}$ 10), which shows the presence of trace amounts of dienes. In the IR spectrum of the methyl esters of the FFAs there were no strong absorption bands in the 900-1000-cm^{-1} region which shows the absence of a trans configuration of the olefinic bonds.

The activity of the total FFAs was 50 units/mg, while the methyl esters of the FFAs possessed no antibiotic activity.

The fatty acids (FAs) of green algae studied up to the present time consist of a mixture of 15-22 substances, the main ones of which are palmitic, stearic, oleic, linoleic, and linolenic acids [10, 11].

According to gas–liquid chromatography (GLC), the FFAs of *Scen. obliquus* UA-2-6 consisted of 20 acids (Table 1). In Table 1 the acids are given in the sequence of their emergence on GLC.

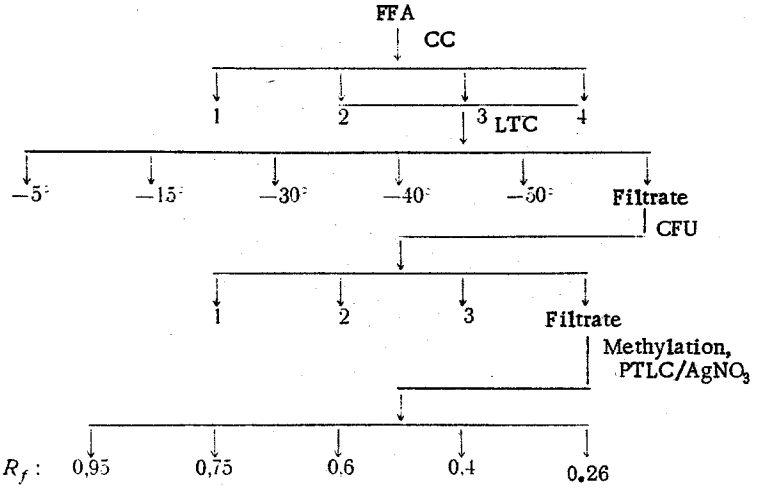
TABLE 1. Composition and Activities of Fractions of the FFAs of *Scenedesmus obliquus* UA-2-6 Obtained by the CC, LTC, and CFU Methods (GLC, %)

Acid	r*	Initial total FFAs	CC: 1	CC: 2–3–4	Filtrate: LTC	Filtrate: CFU
9:0	0.11	0.3	0.3	0.2	0.1	—
10:0	0.18	0.1	0.1	0.1	—	—
11:0	0.22	0.1	0.1	0.1	—	—
12:0	0.33	0.1	0.1	0.1	0.1	—
X_1	0.41	0.2	0.2	0.1	—	—
14:0	0.52	0.6	0.6	0.6	0.3	—
14:1	0.63	0.2	0.4	0.1	0.2	—
15:0	0.78	0.2	0.4	0.1	—	—
16:0	1.00	24.5	26.4	23.0	0.7	—
16:1	1.15	4.1	3.3	4.7	3.3	—
16:2	1.25	1.4	1.2	1.5	1.5	1.3
X_2	1.41	0.4	0.7	0.1	0.1	0.6
16:3	1.60	0.7	1.1	0.3	3.3	5.6
18:0 – X_3	1.77	9.2	5.0	12.7	19.0	62.2
18:1	1.96	20.2	24.3	16.8	19.7	—
18:2	2.33	9.3	10.0	8.7	11.1	1.4
X_4	2.62	0.2	0.1	0.2	0.2	0.2
18:3	2.92	26.0	24.2	27.9	36.1	14.9
X_5	3.30	2.2	1.5	2.7	4.3	13.8
Activity, units/mg		50	Weak	100	200	800

*Relative retention time, C_X/C_{16}.

In order to determine which of the acids composing the total FFAs of *Scenedesmus* possesses antibiotic activity, we tested the following individual acids for activity by the paper disk method: lauric, palmitic, stearic, and arachidic. Because we lacked pure samples of the 18:1, 18:2, and 18:3 acids forming the main unsaturated acids of the FFAs (see Table 1), we subjected to the given test the total acids of cottonseed oil (18:1 — 17.5%; 18:2 — 52%) and the total acids of the seeds of *Ziziphora pedicellata* (18:3 — 68%) [12]. To exclude the influence of active oxidation products, all the samples were also studied by bioautographic manifestation after chromatography on TLC. Under the TLC conditions used, compounds with oxygen-containing groups were clearly separated and did not interfere with the determination of the activity of the acids under investigation. Of the samples investigated, lauric acid possessed a weak activity (60 units/mg), but its amount in the FFAs of the algae (0.1%) is insufficient for the activity to be determined by this acid alone.

To isolate its active components, the FFA fraction of *Scenedesmus* was separated by the following scheme:



Scheme of the fractionation of the antibiotically active components of the FFAs of Scenedesmus obliquus UA-2-6.

The activity of the fraction was due to the acids present on TLC in the lower part of the FFA spot, i.e., even on the sorbent a certain differentiation of the acids took place. This property was used for the preliminary concentration of the active components by column chromatography (CC) on silical gel. Four fractions were obtained of differing composition and activity (see Table 1).

The first fraction possessed almost no activity. In the three other active fractions the amount of 16:0, 18:1, and 18:2 acids was less, but the amount of polyunsaturated acids and also of the acid X_3 not separated from stearic acid under the initial GLC conditions had increased. These three fractions were combined and fractionated further by low-temperature crystallization (LTC). As the temperature of LTC was lowered to −50°C, the antibiotic activity of the fractions rose, which correlated with the increase in the proportion of polyunsaturated fatty acids of the C_{16} and C_{18} series in their composition. The greatest activity was possessed by the filtrate (see Table 1).

The further fractionation of the acids from the LTC filtrate was carried out by complex-formation with urea (CFU). Three fractions of acids forming complexes, and a filtrate, were obtained.

The most active filtrate was enriched mainly with three acids, of which the principal one was the X_3 acid (62.2%). It was found from the results of the biological tests that linoleic and linolenic acids showed no activity. Consequently, the activity of the CFU filtrate is due to the acids X_3 and X_5.

To isolate the acids X_3 and X_5 we used preparative TLC (PTLC) on silica gel with the addition of $AgNO_3$ in system 1. The sum of the acids of the filtrate was previously converted into the methyl esters by methylation with diazomethane. Fractions with R_f 0.95, 0.75, 0.6, 0.4, and 0.26 were obtained.

The fraction with R_f 0.95 was identified on the basis of GLC and NMR results. According to GLC, this fraction contained the peaks of the components that we had not identified. In the NMR spectrum of the fraction signals characteristic for phthalic acid esters appeared in the 2.56, 5.94, 8.75, and 9.14 ppm regions [12]. The compositions of the acids of the other fractions obtained by the PTLC method are given in Table 2. As can be seen from this table, the fraction with R_f 0.26 is the most active of the fractions obtained and contains the acid X_3 with a small amount of X_5. By paper chromatography with bioautography it was established that both acids possessed activity.

The IR spectrum of the methyl ester (ME) of the acid X_3 had no strong absorption in the 900-1000-cm^{-1} region and, therefore, the olefinic bonds in the acid do not have the trans configuration.

The UV spectrum of the fraction has no absorption at 220-360 nm, which shows the absence of conjugated double bonds in X_3.

The NMR spectrum of the methyl ester of X_3 (Fig. 1; τ scale) showed: the signal of the olefinic protons of an isolated double bond (4.76 ppm, miltiplet, 8H, J = 4 Hz), the signal of a methoxy group (6.46; singlet, 3H), the signal of a diallylic methylene group $=CHCH_2CH=$ (7.24; multiplet, 6H), a complex signal consisting of an apparent doublet of the protons of a $=CH_2CH_2CH_2COO$ group and a multiplet of the protons of a $CH_3CH_2CH=$ group (7.7-8.2 ppm, 6H), and the signals of the protons of $CH_3CH_2CH=$ group (9.06 ppm, resolved triplet, J = 8 Hz). The remaining signals in the spectrum were assigned to the X_5 impurity.

The nature of the signal of the olefinic protons shows the isolated nature of the four double bonds in the acid X_3. The complex signal in the 7.7-8.2-ppm region is analogous to the signal observed in the case of the location of one of the olefinic bonds on a quaternary carbon atom [13]. From the degree of resolution of the signal of the protons of the methyl group it may be concluded that there is no double bond in the β position.

From the absence in the IR spectrum of absorption bands characteristic for the trans configuration of olefinic protons, and from the value of the coupling constants of the olefinic protons it may be concluded that all the double bonds in the acid X_3 have the cis configuration [13, 14].

Thus, acid X_3 has the structure of cis-hexadeca-4,7,10,13-tetraenoic acid (I)

$$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH_2COOH.$$

This structure was confirmed by the production of propionic and succinic acids when the fraction was oxidized by the periodate–permanganate method.

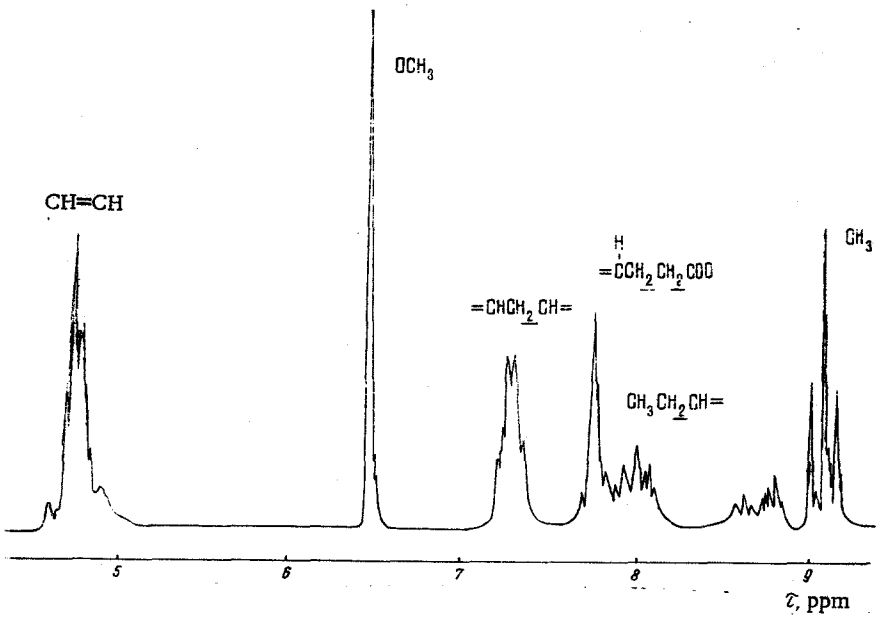


Fig. 1. NMR spectrum of the methyl ester of cis-hexadeca-4,7,10,13-tetraenoic acid.

TABLE 2. Compositions and Activities of Fractions of Unsaturated Acids of the FFAs of *Scenadesmus obliquus* UA-2-6 Obtained by the PTLC Method (GLC, %)

Acid	Fraction with R_f			
	0.75	0.6	0.4	0.26
14:1	5.3	—	—	—
16:1	42.9	—	—	—
16:2	—	25.2	—	—
X_2	—	3.9	4.3	—
16:3	—	5.5	23.5	—
X_3	—	2.7	14.7	89.4
18:1	51.8	—	—	—
18:2	—	2.8	0.6	—
X_4	—	5.7	8.2	—
18:3	—	54.2	37.2	—
X_5	—	—	11.5	10.6
Activity, units/mg	None	Weak	300	1200

The structure of acid X_5 was not determined, but according to preliminary results it is a tetraenoic acid of the C_{18} series.

The presence of a tetraenoic acid of the C_{16} series in the lipids of *Chlorella* was first reported by Paschke et al. [15]. On the basis of the results of a study of the UV spectra of isomerized crude fractions of the fatty acids these authors gave the assumed structure (I). Subsequently, the acid (I) was identified by the same method in the lipids of the herring [16] and of *Scenedesmus* [17]. However, other authors have not detected (I) in the lipids of algae at all [18, 19]. This acid has not previously been isolated in the individual form and its antibiotic properties have not been studied. It is possible that the absence of just this acid explains why in some cases cultures of algae show no antibiotic properties [20].

We isolated the cis-hexadeca-4,7,10,13-tetraenoic acid from the biomass of *Scen. obliquus* UA-2-6 in an amount of 2.5% of the total FFAs of the lipid extract.

Tests on a number of cultures of fungi, yeasts, and bacteria showed that the acid has a broad spectrum of antibiotic action, but its greatest activity is shown on yeast and Gram-positive bacteria. This confirms the results that we obtained previously for unpurified preparations of the lipid fractions from *Scenedesmus* [7].

EXPERIMENTAL

The UV spectra were taken on an SF-16 instrument in hexane, the IR spectra on a UR-10 instrument in a film, and the NMR spectra on a Varian XL-100 instrument in CCl_4 with TMS as internal standard. The gas–liquid chromatograms were obtained on a UKh-2 instrument under the conditions described previously [21].

As the sorbent for CC we used type ShSK silica gel, 100-200 μ, treated by a published method [22]. Thin-layer chromatography was performed on "Chemapol" type L 5/40 μ silica gel with the addition of 13% of gypsum in systems 1) hexane–diethyl ether (70:30) and 2) chloroform. Preparative TLC was performed on L 5/40 μ silica gel with the addition of 10% of gypsum and 10% of $AgNO_3$ in system 1 [21], and paper chromatography in the CH_3COOH–$HCOOH$–H_2O (3:1:0.1) system on "Filtrak" No. 8 paper impregnated with a 10% solution of paraffin oil in benzene [23].

The antibiotic activities of the fractions were determined on samples of free acids by the method of paper disks on agarized peptone–maize medium and by the serial dilution method in Sabouraud medium [24]. Bioautography was carried out by the method described by Betina [25].

The local strain UA-2-6 of the algae *Scen. obliquus* was cultivated on medium 04 for 12-14 days [26]. The biomass was separated by centrifuging and was dried in the shade. The air-dry material (1.5 kg) was comminuted and was extracted with diethyl ether at room temperature.

The extract obtained was chromatographed on a column in system 1. The fractions containing FFAs were combined, the solvent was distilled off, the residue was dissolved in diethyl ether, the solution was treated with 5% Na_2CO_3 solution, and the nonacid impurities were removed with ether. The soaps were decomposed under a layer of ether with 10% H_2SO_4. The acids were extracted with ether. The *Scenedesmus* FFAs had a faint yellow color and consisted of a viscous liquid which solidified at 0°C. The amount of FFAs obtained was 27.5 g (1.8% of the air-dry biomass).

Rough fractionation of the FFAs was carried out on a column in system 1, 200-ml fractions being collected. The combined fractions 2, 3, and 4 (15.6 g) were subjected to LTC from 5% solution in acetone, precipitates being collected at temperatures of −5, −15, −30, −40, and −50°C. The filtrate (5.1 g) was separated further by the CFU method. Complex-formation was carried out at a ratio of methanol to sample to urea of 40:5:15. After the removal of the first complex, 8 g of urea was added to the solution, and after the removal of the second another 4 g was added. The filtrate obtained after three stages of complex-formation (1.1 g) was methylated with diazomethane, and 220 mg of the methyl esters of the acids of the CFU filtrate were separated by the PTLC method on silica gel with the addition of $AgNO_3$. This gave 80 mg of tetraenoic fraction.

Oxidative degradation was carried out with the periodate—permanganate reagent [27]. Thin-layer chromatography of the mixture of fragments from the oxidation of the tetraenoic fraction in the form of the ammonium salts was performed on cellulose: glass plates 6 × 9 cm; solvent systems: ethanol—ammonia—water (20:3:2) and tert-butanol—ammonia—water (25:3:5).

SUMMARY

By the method of checking antibiotic activity, conditions have been selected for the isolation of cis-hexadeca-4,7,10,13-tetraenoic acid from the fatty-acid fractions of the lipids of *Scenedesmus obliquus* UA-2-6. Its structure has been confirmed and it has been established that this acid possesses a broad spectrum of antibiotic action.

LITERATURE CITED

1. E. Jorgensen. Physiol. Plantarum, 9, 712 (1956).
2. A. L. Shinkarenko and M. I. Mamaichuk, in: The Study and Use of the Medicinal Plant Resources of the USSR [in Russian], Leningrad (1964), p. 433.
3. K. Balak, O. Rydlo, and M. Vojta, in: Lectures at the Tenth Coordination Conference on Subject 1-18.4 of Scientific and Technical Cooperation of Comecon, Trebon, Czechoslovakia [Russian version] (1975), p. 283.
4. N. Garcia de Martinez Nadal, L. V. Rodriguez, and C. M. Casillas Chapel, Antimicrob. Agents Chemother., 68 (1964).
5. G. Blaauw-Jansen, Nature (London), 174, 312 (1954).
6. E. Jorgensen, Physiol. Plantarum, 15, 530 (1962).
7. A. A. Akhunov and T. T. Taubaev, Uzv. Biol. Zh., No. 5, 6 (1975).
8. A. A. Akhunov and Kh. F. Yakubov, in: Physiological and Biochemical Aspects of the Cultivation of Algae and Higher Aquatic Plants in Uzbekistan [in Russian], Tashkent (1976), p. 84.
9. J. A. Williams, A. Sharma, L. J. Morris, and R. T. Holman, Proc. Soc. Exp. Biol. Med., 105, 192 (1961); J. J. Biezenski, W. Pomerance, and J. Goodman, J. Chromatogr., 38, 148 (1968).
10. M. Matucha, L. Zilka, and K. Švihel, J. Chromatogr., 65, 371 (1972).
11. G. L. Klyachko-Gurvich, V. E. Semenenko, and A. G. Vereshchagin, Biokhimiya, 35, 808 (1970).
12. S. D. Gusakova and A. U. Umarov, Khim. Prirodn. Soedin., 551 (1975).
13. C. Y. Hopkins, Progr. Chem. Fats Other Lipids, 8, No. 2, 215 (1965); D. J. Frost and F. D. Gunstone, Chem. Phys. Lipids, 15, 53 (1975).
14. J. Brand and G. Eglinton, Applications of Spectroscopy to Organic Chemistry, Oldbourne Press, London (1965).
15. R. F. Paschke and D. L. Wheeler, J. Am. Oil Chemists' Soc., 31, 81 (1954).
16. W. Stoffel and E. H. Ahrens, J. Am. Chem. Soc., 80, 6604 (1958).

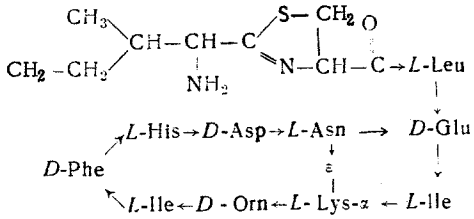
17. E. Klenk and W. Knipprath, Z. Physiol. Chem., 317, 243 (1959).
18. G. W. Patterson, Lipids, 5, 597 (1970).
19. H. Schlenk, H. K. Mangold, J. L. Gellerman, W. E. Link, R. A. Morissette, R. T. Holman, and H. Hayes, J. Am. Oil Chemists' Soc., 37, 547 (1960).
20. G. R. Vela and C. N. Guerra, J. Gen. Microbiol., 42, 123 (1966).
21. S. D. Gusakova and A. U. Umarov, Khim. Prirodn. Soedin., 27 (1972).
22. A. A. Akhrem and A. I. Kuznetsova, Thin-Layer Chromatography [in Russian], Moscow (1964), p. 16.
23. É. I. Gufienova, A. U. Umarov, and A. L. Markman, Maslob-Zhir. Prom., No. 9, 34 (1969).
24. V. S. Dmitrieva and S. M. Semenov, The Microbiological Control of the Activity of Antibiotic Preparations [in Russian], Moscow (1965), pp. 28, 72.
25. V. Betina, Antimicrob. Agents Chemother., 637 (1966).
26. A. M. Muzafarov and T. T. Taubaev, Chlorella [in Russian], Tashkent (1974), p. 43.
27. S. D. Gusakova, A. L. Markman, and A. U. Umarov, Maslob-Zhir. Prom., No. 4, 21 (1969).

BACITRACIN AND GRAMICIDIN S AS INHIBITORS OF CARBOXYLIC PROTEINASE

V. M. Stepanov, M. V. Gonchar, and G. N. Rudenskaya

UDC 577.156

The antibiotic bacitracin — a mixture of cyclic polypeptides of similar structure — is produced by *Bac. licheniformis*. Below we give the structure of the main representative of the family of bacitracins — bacitracin A [1]:



Makinen [2] investigated the action of bacitracin on a number of proteolytic enzymes. It was found that it inhibits the papain-catalyzed hydrolysis of N_α-benzoyl-D,L-arginine 2-naphthylamide and the subtilisin-, papain-, and leucine-aminopeptidase-catalyzed hydrolysis of L-leucine 2-naphthylamide. The K_I values for the inhibition of papain, subtilisin, and leucine aminopeptidases by bacitracin are 5.0, 4.5, and 2.0 mM, respectively. On the other hand, bacitracin shows no capacity for inhibiting aminopeptidase B and trypsin, and α-chymotrypsin is inhibited feebly. In view of the fact that bacitracin has proved to be a suitable ligand for the affinity chromatography of carboxylic proteinases, we have studied its capacity for inhibiting a typical enzyme of this class — porcine pepsin.

As the pepesin substrate we selected a protein substrate — hemoglobin. The proteolytic activity was determined by a modification of Anson's method [3]. It was found that the dependence of the reciprocal rate of cleavage ($1/\Delta E_{280}$) on the reciprocal concentration of hemoglobin ($1/[S_0]$) obeys a linear law at least in the range of concentrations of substrate of from 1 to 20 mg/ml. A graphical calculation of the apparent value of K_M for the hydrolysis of hemoglobin by pepsin (Fig. 1, line a) gives a value of 5.7 mg/ml or 0.35 mM (calculated to one hemoglobin subunit).

Treatment of the kinetic results on the cleavage of hemoglobin by pepsin in the presence of bacitracin at various concentrations of the substrate and inhibitor in the Lineweaver–Burk coordinates (see Fig. 1, lines a, b, and c) shows that the dependence of $1/\Delta E_{280}$ on $1/[S_0]$ has the form of a family of straight lines intersecting on the axis of ordinates. Consequently bacitracin inhibits porcine pepsin as a fully competitive, or close to fully competitive, inhibitor. Analysis of the kinetic results on inhibition by the simplified Dixon method (Fig. 2) in the coordinates $1/\Delta E_{280}$ versus [I] gives for K_I a value of 3.1 mg/ml or 2.3 mM. Thus, bacitracin inhibits pepsin with approximately the same efficiency as papain, subtilisin,

M. V. Lomonsov State University. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 385-389, May-June, 1978. Original article submitted December 21, 1977.

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