12/28

INFORMATION REPORT INFORMATION REPORT

CENTRAL INTELLIGENCE AGENCY

29 DEC 1965 (b)(3)

CONTROLLED DISSEM ~~CONFIDENTIAL~~ NO DISSEM ABROAD

COUNTRY USSR

REPORT NO. (b)(3)

SUBJECT Current Research Projects and Problems of the Institute of Chemistry of Natural Products, Moscow/Intense Sovbloc Interest in Streptothrycin, a Toxic Substance with Possible BW Implications

DATE DISTR. 23 December 1965

NO. PAGES 2

REFERENCES (b)(3)

DATE OF INFO. to Nov 65

PLACE & DATE ACQ. --- to Nov 65

THIS IS UNEVALUATED INFORMATION

SOURCE:

1. During his visit to US microbiological facilities in October and November 1965 Dr Mikhail M Shemyakin, an active member of the Soviet Academy of Sciences and director of the Institute of Chemistry of Natural Compounds, Moscow, indicated his interest in the development of US mass spectrometry of natural products. He expressed a genuine surprise when he learned that even major US microbiological research laboratories lacked mass spectrometers, and boasted that his institute had four normal spectrometers and one high resolution mass spectrometer. (The latter costs about US$200,000.) (b)(1) (b)(3)

2. According to Shemyakin, his institute is engaged among others, in the following research projects:

   a. Application of mass spectrometry to amino acid sequence analysis in polypeptides.

   b. Classification of actinomyces using chemistry of the cell wall.

   c. Biological effects of DNA and its breakdown products.

   d. Research on streptothricin.

3. To underline his vivid interest in streptothricin, Shemyakin brought with him a hitherto unpublished paper written by Professor A S Khokhlov, deputy director, and his co-worker, Dr P D Reshetov, Institute of the Chemistry of Natural Products. Shemyakin stated that Khokhlov and his associates have been working on streptothricin, a highly toxic substance, for several years and that they needed US streptothricin "for comparison."

4. As is known, streptothricin was first reported by US investigators in

5 4 3 2 1 S-NO U-NO 5 4 3 2 1

~~CONFIDENTIAL~~

GROUP 1 Excluded from automatic downgrading and declassification

INFORMATION REPORT INFORMATION REPORT

1940. After two years of research, streptothricin was declared nephrotoxic and it was agreed that further study would be useless. However, the researchers also found that many peptide-like antibiotics related to streptothricin have varied biological activities against both Gram-positive and Gram-negative bacteria, fungi and viruses. Since some US microbiologists felt that various members of the streptothricin family of antibiotics may be found non-toxic and may prove to be highly important antibiotics, research has not entirely been abandoned.

5. Judging from private requests made by Soviet, Czechoslovak and Hungarian microbiologists visiting in the US during the past few years, there appears to be a concerted effort on the part of Sovbloc researchers to obtain samples of streptothricin from their US colleagues. US microbiologists recall that a Sovbloc visitor indicated his interest in streptothricin in connection with his research in the field of equine encephalitis. Several US researchers are convinced that the Soviet and satellite work on streptothricin has BW implications.

6. Shemyakin's institute is not doing any work on ergot alkaloids. However, a new research organization, the Institute of the Biochemistry and Physiology of Microorganisms, Soviet Academy of Sciences, now located at Ul Vavilova 18, Moscow, but scheduled to move into its own building in 1966, is conducting some work on ergot alkaloid production. The director of the new institute is Dr Nikolay Yerusalimskiy, a former deputy director of the Institute of Microbiology, Moscow. Ierusalemskiy's deputy is Dr Georgiy K Skryabin.

7. The work of the Institute of Chemistry of Natural Products is determined by the following criteria:

   a. The research problem has to be interesting from a biological point of view.

   b. The problem has to be of chemical interest from the point of view of chemistry of new products.

   c. The problem has to have practical biological implications since funds allocated for fundamental research are limited.

8. The institute's major efforts now center around natural product research for which more funds are available than for work on synthetic drugs.

9. Shemyakin's institute has only a small, insignificant project concerning Soviet "popular" medicinals, and as a rule, Soviet microbiologists have a low opinion of Chinese Communist research efforts in this field.

10. One of the top priorities of Shemyakin's institute is research on interferon. Soviet researchers are of the opinion that interferon which has been investigated by several US scientists could be important in virology as an antiviral and, possibly an antitumor agent. Some Soviet scientists believe that interferon may be involved in the prevention of tumor. However, they admit that they have been hindered by assay problems. Shemyakin requested several US researchers doing work on interferon to help his institute with the solution of the assay problem.

end

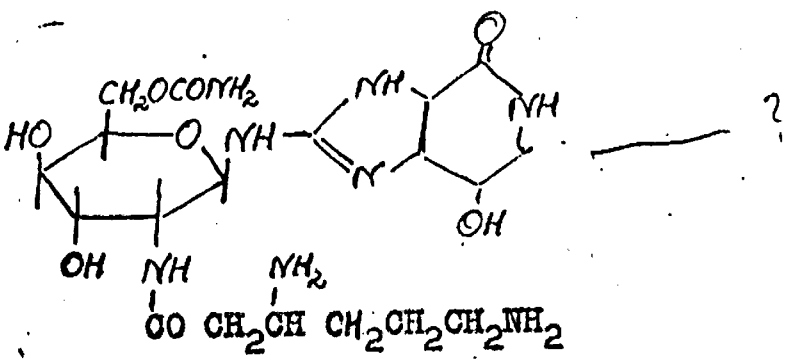
THE PRESENT STATE OF INVESTIGATION OF STREPTOTHRICIN ANTIBIOTICS

Prof.A.S.Khokhlov and P.D.Reshetov
Institute for Chemistry of Natural Products,
USSR Academy of Sciences, Moscow, USSR.

Microorganisms producing streptothricin and related antibiotics are very widespread in nature. Substances belonging to this class are frequently encountered by all engaged in screening tests for new antibiotics from actinomycetes.

The high antibiotic activity of the streptothricins and their delayed toxicity which becomes apparent only on prolonged administration have time and again attracted the attention of the research workers to these compounds. Streptothricin - the parent compound of this group and its best known member was discovered by S.Waksman and H.B.Woodruff in 1942. Later a number of ~~scarcely~~ partly purified antibiotic preparations with properties similar to the streptothricin was described. However in the majority of cases the ~~authors~~ investigators confined themselves only to general description of nonhomogeneous preparations to which they gave new names or ~~indexes~~ numbers (usually on insufficient grounds). ~~In virtue~~ Because of this to the present time streptothricin antibiotics have been given ~~count~~ number about 70 ~~names~~, which, however, as our studies have shown are without preparation a rational basis.

The structure of streptothricin was established in 1961-1963 as ~~the~~ a result of extensive work by American and English groups ~~of~~ scientists.



It is noteworthy that streptomycetes produce streptothricin free of other antibiotics of the same group; this greatly facilitates its isolation and purification.

Almost simultaneously proposals were made concerning the structures of streptolin, (H.E.Carter, E.E.van Tamelen), racemomycin O (S.Takemura), and partially of roseothricin A (Goto et al) and geomycin (H.Brockmann). However, our investigations have shown, that these structures are either inaccurate, or (as in the case of racemomycin O) lacking of sound basis. This is probably due to the fact that the preparations studied were mixtures rather then individual compounds.

It must be emphasized, that the isolation of individual streptothricins from crude preparations was for a long a very difficult task. All spreptothricins are insoluble in nonpolar solvents (practically they are soluble only in water), they are unstable in acid and alkaline solutions, and at elevated temperatures also in a neutral medium; they display no characteristic bands in the UV-spectra and so on. We have found, that different streptothricins, when obtained in the individual state, are almost indistinguishable from each other in many of their properties - for example, with the exception of only the most simple streptothricin F, i.e. streptothricin itself, they have

the same IR - spectrum. All this greatly limits the possibilities of the investigator in selecting methods for fractionating the streptothricins. Preparative partition chromatography on cellulose in butanol-pyridine-acetic acid-water (15:10:3:12) solvent system used, for instance, in the separation of the components of racemomycin and antibiotic A-8265 proved to be very laborous and of little efficiency and did not receive wide application. Hence the first stage in our investigations was the elaboration of methods for the isolation and purification of individual streptothricins, on a preparative scale.

The different basicities of streptothricin and streptolin led us to the assumption that, perhaps, the other streptothricins differ from each other in the number of free amino groups. On these grounds we believed ion-exchange chromatography might prove to be a suitable method for fractionating of the streptothricin preparations. For rational selection of the fractionation conditions we first determined the distribution coefficients (K) of one of our crude preparations-grisin (grisemin)-between the carboxylcontaining ion-exchange resins Amberlite IRC-50 and carboxymethylcellulose (the sodium form) and solutions of sodium chloride and sodium acetate of various concentrations. The optimal K values were found for adsorption of the antibiotic on carboxymethylcellulose from 0.2-0.4 molar solutions of sodium chloride.

See Fig. 1

The analytical experiments (with a column 0.9cm diameter and 40 cm long) showed that under such conditions this method, was highly efficient.

Fig. 2

By these means all six antibiotics which were found to be simultaneously present in the mixture, could be reliably separated.

Thus, ion-exchange chromatography on carboxymethylcellulose in a sodium chloride concentration gradient formed the basis for a general scheme we developed for the isolation and purification of streptothricins; and in this way we were able to overcome obstacles presented by the similarity of the properties of these antibiotics.

The operations used in each stage of the purification are given in the following scheme, but some of them require explanation.

Stage in the isolation and purification of individeal streptothricins.

Principial stages in the purification process	Corresponding operations.
1. Preliminary purification of crude preparations	1. Treatment with active charcoal
	2. Preparation of picrate and its transformation into hydrochloride
2. Fractionation of crude preparations	1. Ion exchange chromatography on carboxymethylcellulose
3. Isolation of individual streptothricins from eluates	1. Adsorption on Amberlite IRC-50
	2. Desalting of ion exchange resin.
	3. Desorption and drying of antibiotics
4. Further purification of obtained compounds	1. Precipition as the picrate and transformation into the hydro chloride

2. Fractional precipitation

3. Preparation of pure sulphates and oxalates.

After isolation from cultural broth streptothricin preparations usually contain pigmented impurities and certain amounts of inorganic salts so that they must be subjected to a preliminary purification.

Fig.3. The results of the chromatography of four preparations at optimal charge of the column (grisemin - 5 mg/ml, phytobacteriomycin - 2 mg/ml, polymycin and antibiotic № 4714-12-1, 5 mg/(ml) are given on the next slide.

Because the qualitative and quantitative composition of the crude streptothricin mixtures depends on the fermentation conditions, the fractionation was sometimes repeated, as a rule using for the first fractionation maximal charges of columns, for example for polymycin up to 6 mg/ml.

The fractions were analysed by measuring the optical density at 215 mμ, which is a linear function of the concentration of the compounds. After fractionation the separated streptothricins were dissolved in 3-5 liters of 0.2-0.3 molar solutions of sodium chloride, i.e. the ratio by weight of antibiotic to sodium chloride thus being about 1 to 50. Concentrating and desalting were achieved by adsorption on the sodium forme of Amberlite IRC-50. However the preparations obtained at this stage still contained small amounts of sodium chloride and traces of inactivation products. The neutral sulphates and oxalates of the streptothricins were prepared by means of thoroughly washed

finely ground (400-600 mesh) of anion-exchange resin Amberlite IRA-400 in the appropriate (sulphate or oxalate) form.

As a result 18 chromatographically pure streptothricins belonging to six different types of compounds were obtained from five crude preparations - polymycin, phytobacteriomycin, Japanese antibiotic racemomycin, grisemin and antibiotic № 4714-12

Fig. 4

All compounds, belonging to the series A to E have the same characteristic IR-spectrum with strongly marked amide bonds: 3250, 1658, 1563 and 1310 cm^{-1}. The IR -spectrum of streptothricins belonging to F-type differs only by the absence of absorption band in the 1563 cm^{-1} region. Table 1 gives the optical activity $[\alpha]_D$ of the majority of the compounds obtained.

Table 1.

Optical activity of streptothricin antibiotics

Name of initial preparation	$[\alpha]_D$ of streptothricin hydrochlorides (c 0.9 in methanol)					
	A	B	C	D	E	F
Polymycin	-9,2	-9,6	-8,5			
Phytobacteriomycin		x)	-12,8	-21,6		x)
Grisemin				-21,0	x)	-35,7
Antibiotic № 4714-12			-12,2	-22,0	-36,6	-34,4 -42,0
Streptothricin				-22,0 xx)		
Streptolin						

x) These compounds were obtained in small amounts and their $[\alpha]_D$ were determined only approximately.

xx) Data from the literature.

A very important characteristic of the streptothricin compounds is the number of free amino groups in their molecules, which determines their basicity. The determining the number of free amino groups we made use of a method described in the literature based on the electrophoresis of N-3,5-dinitrosulphophenyl derivatives (DNSP) of different degress of substitution. The use of DNSP-derivatives for this purpose has the advantage over that of DNP-derivatives in high solubility of the DNSP-derivatives in aqueous electrolytes and in increase of 2 units of charge for each substitution. However, preliminary experiments revealed that the interaction of potassium 4-chloro-3,5-dinitrobenzenesulphonate with amino groups in the presence of triethylamine was accompanied by partial inactivation of the streptothricins and led to extra spots on the electrophoregrams. In analysing the electrophoregrams these extra spots could be excluded by comparing the mobility DNSP-derivatives of the six types of streptothricins with that of their inactivation products obtained by treating antibiotics with dilute mineral acids.

Fig 5.

It was therefore possible to interpret the electrophoregrams of DNSP-derivatives of streptothricins as follows. The streptothricin, with two free amino groups (streptothricin F) can, however, take up three equivalents of acid. Therefore its DNSP-derivatives on electrophoresis in acid electrolytes must

have charges of +1 and -1 rather then 0 and -2. And in fact these derivatives are readily noticeable as two intensely coloured spots, simmetrically situated on both sides of the zero-line.

These conclusions were of essential importance in analyzing of the electrophoregrams of DNSP-derivatives of the other antibiotics. In this way it became possible to determine the number of free amino groups and the basicity of all six types of streptothricins (see Table 2)

Table 2.

Electrophoresis of DNSP-derivatives of Streptothricins

Type of streptothricin	DNSP-derivatives of the streptothricin charges and location of the latter on the electrophoregrams	Number of amino groups	Basicity of streptothricins
	− +		
F	+1 -1	2	3
E	+2 0 -2	3	4
D	+3 +1 -1 -3	4	5
C	+4 +2 0 -2 -4	5	6
B	+5 +3 +1 -1 -3 -5	6	7
A	+6 +4 +2 0 -2 -4 -6	7	8

Determination of the basicity permitted a more detailed characteristic of the predominant components in the crude preparations. The very hygroscopic amorphous hydrochlorides of these compounds were transformed into neutral sulphates and oxalates by means of the appropriate form of the anion-ex-

change resin Amberlite IRA-400. Being somewhat less hygroscopic these salts still strongly retain polar solvents, which considerably effects the analytical data. Drying at elevated temperatures (about 80°-100°) of the streptothricin salts (expecially of the sulphates) is excluded because of their instability.

The equivalent weights of many streptothricins were determined by oxidimetric titration of oxalic acid, preliminarily precipitated as the calcium salt. The results then allowed us to determine the molecular weights and empirical formulas of a number of antibiotics (see Table 3).

Table 3.

Some properties of the streptothricins.

Antibiotics	Melting points °C (with decomposition)		Molecular weights of oxalates		Empirical formulas of the streptothricin oxalates
	oxalates	sulphates	found	calculated	
Phytobacteriomycin F	178,5-180				
Antibiotic №4714-12 F	179-181				
Grisemin F	179-181		654	655	$C_{19}H_{34}N_8O_8 \cdot 1,5\,C_2H_2O_4 \cdot H_2O$
Phytobacteriomycin D	184-186	214-215	1040	1001	$C_{31}H_{58}N_{12}O_{10} \cdot 2,5\,C_2H_2O_4 \cdot H_2O$
Grisemin D	184-186		1015	1001	$C_{31}H_{58}N_{12}O_{10} \cdot 2,5\,C_2H_2O_4 \cdot H_2O$
Phytobacteriomycin O	183,5-185	215-217	1176	1174	$C_{38}H_{70}N_{14}O_{11} \cdot 3\,C_2H_2O_4 \cdot H_2O$
Polymycin B	181-183	216-217	1393	1347	$C_{43}H_{82}N_{16}O_{12} \cdot 3,5\,C_2H_2O_4 \cdot H_2O$
Polymycin A	181-183	216,5	1496	1520	$C_{49}H_{94}N_{18}O_{13} \cdot 4\,C_2H_2O_4 \cdot H_2O$

Qualitative determination of the components in the hydrolyzates (6n.HCl, 105° ± 1°, 24 hours) of representatives of the six types of streptothricins was carried out by radial chromatography in four solvent systems: 1) butanol-formic acid-water (75:15:10); 2) butanol-acetic acid-water (10:2:4); 3) butanol-pyridine-acetic acid-water (15:10:3:12); 4) methyl ethyl ketone-propionic acid-water (30:10:12). Four ninhydrin-positive compounds were found in the hydrolyzates of all six antibiotics. These compounds (in the order of increasing values of Rf) were identified as streptolydin, L-ß-lysin, hexosesamin (probably, α-D-gulosesamine) and 1,6-anhydrohexosesamine by comparison with the hydrolyzate of streptothricin and with authentic samples of L-ß-lysin and streptolydin, as well as by characteristic colour reactions (Elson-Morgan, Weber and others). In the light of these data and the different basicity of the six types of streptothricins the only reasonable explanation of these observations is that different antibiotics have different ratio of residues of these compounds in the molecules. To be more exact the streptothricins may differ, for example, in the number of diamine acid residues as in streptothricin with one L-ß-lysine residue and streptolin, which according to American investigators contains two residues of this acid.

We determined the ratio of amino acid and carbohydrate residues in different streptothricins by the automatic amino acid analyser according to a similar to the method, described by Kominz for the analysis of complex mixtures of ninhydrin-positive compounds, including amino sugars. Preliminarily streptolydine L-ß-lysine and 1,6 - anhydrohexosesamine were

isolated as the hydrochlorides from a hydrolyzate of phytobacteriomycin by partition chromatography on [illegible]cellulose in system I. Analysis of the fractions was performed by horizontal paper chromatography in system two at elevated temperature. The compounds obtained were characterized by comparison with authentic samples and by colour reactions. The calibration of chromatographic column by means of reference substances is shown on slide 8. Analytical data for the hydrolyzates of the six streptothricins are given on slide 9.

Fig. 6
Fig. 7

The amino acid and carbohydrate composition calculated for these six antibiotics are given in Table 4.

Table 4.

Compositions of six types of streptothricins

Cleavage products	Number of residues per 1 mol of streptothricin											
	F		E		D		C		B		A	
	I	II	I	II	I	II	I	II	I	II	I	II
L-ß-Lysin	1,0	1	2,0	2	3,0	3	4,0	4	5,0	5	6,0	6
Streptolydin	0,63	1	0,83	1	0,86	1	1,05	1	0,87	1	1,07	1
Ammonia	0,73	1	0,71	1	0,88	1	0,97	1	1,08	1	1,11	1
Total sugars	0,418	1	0,463	1	0,516	1	0,580	1	0,613	1	0,680	1
Total sugars (per streptolidine residue)	0,66		0,56		0,60		0,55		0,70		0,63	
1.6-Anhydrohexosamine (per hexosamine residue)	2,11		1,96		1,99		1,98		2,08		2,10	

I -Experimental (numbers) of residues

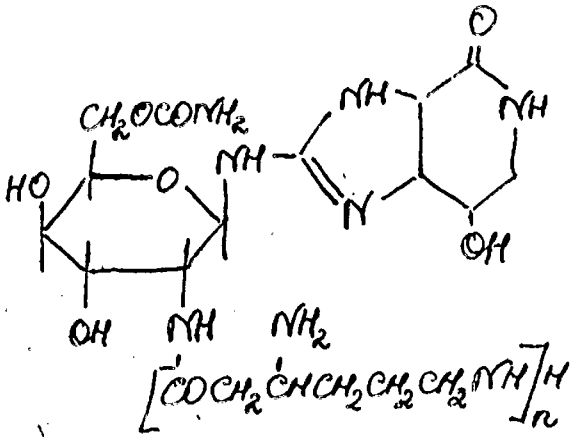
II-Rounded off number

As a result of these investigations the part of hexosamine transformed by hydrolysis into the anhydro-derivative was determined. The constant value of this part for all the antibiotics investigated by us testifies to a certain extent to the identical nature of the amino sugar in these components.

It is seen from Table 4, that all the streptothricins in question gave practically the same ratio (0,6) of hexosamine and streptolidine residues, which is evidence of a constant (circa 55%) yield of amino sugars in the hydrolysis of each antibiotic. Since in the hydrolysis of streptothricin, which contains one mole of amino sugar the yield is the same (about 54%) it has been concluded that the antibiotics all contained one hexosamine residue per mole.

The composition of the six types of strpptothricins given in Table 4 confirms the earlier deduced empirical formula of streptothricin and satisfactorily explains the monotonous alteration in the properties of the other antibiotics of this group. On the basis of these data we may draw the conclusion that the antibiotic streptolin which according to its properties belongs to type D must contain three rather then two L-ß-lysine residues, as had been assumed by American and English investigators and that it should have the empirical formula $C_{31}H_{58}N_{12}O_{10}$ rather then $C_{25}H_{46}N_{10}O_{9}$. Hence the results obtained and data from the literature concerning the sequnce of the moieties of the streptothricins led us to the conclusion that

the structure of all six types of streptothricins may be expressed by the following general formula:



F n = 1
E n = 2
D n = 3
C n = 4
B n = 5
A n = 6

Based on the chromatographic investigation of over twenty streptothricin preparations and comparison of the results with reported data we concluded that all the described crude preparations of streptothricin antibiotics contain the six above-mentioned compounds in different proportions. We propose to name this six components; streptothricin A, Streptothricin B, C, D, E and F. Thus pleocidin (USA) and racemomycin (Japan) consist of streptothricins D, E and F, geomycin (West Germany)- of B, C and D, nurseothricin (East Germany) of C and D, virothricin (East Germany) - of D and F.

Fig. 8

The distribution of different streptothricins in nature is given in Table 5.

Table 5.

Distribution of streptothricins in nature

Types of streptothricins based on the Rf values	Percentage of preparations, containing more then 7% of component	Percentage of component in preparation
A	13	7-10[1]
B	37	10-20[2]
C	53	10-25

D	90	30-60
E	33	10-15[3)]
F	52	15-50[4)]

1) Excepting polymycin which contains about 40% of this component.

2) Excepting polymycin and geomycin which contain ~~which con-~~ tain 40% and 30% of this componnnt, respectively.

3)Excepting pleocidin and racemomycin which contain about 30% of component E

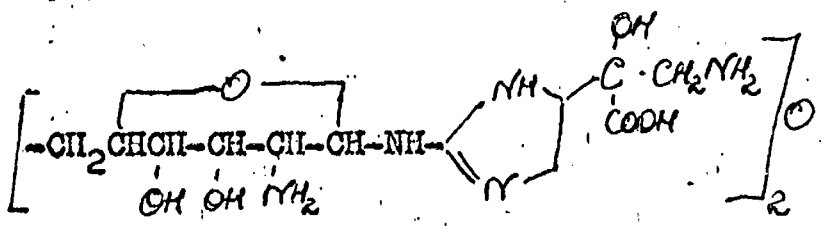
4)Streptothricin is 100% component F.

The question about the chemical nature of racemomycin O is not so clear, because according to published data it differs from other streptothricins in containing instead of gulosamine, glucosamine and also racemonic aldehyde. Professor Taniyama ~~from~~ Nagasaki University told me during ~~my~~ visit to his laboratory, that the ~~producing~~ strain lost the ability to produce this compound, so that straightforward reinvestigation of its properties was not possible. It is not fully excluded, that racemomycin O is not an individual substance, but a mixture of streptothricins.

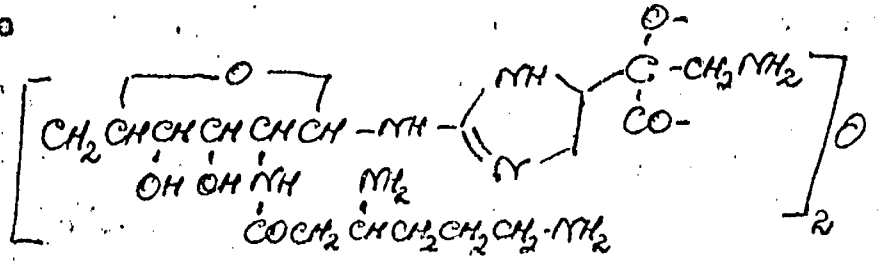
Special attention is merited by the antibiotic roseothricin A, which we believe to be identical to streptothricin.

According to Japanese authors this antibiotic was separated from the accompanying roseothricins B and C by counter current distribution in the system n-butanol - 5% toluenesulphonic acid. For long it was assumed to be an individual substance. The empirical formula of roseothricin A was determined as $C_{38}H_{65}N_{15}O_{16}$. 6HCl. Its acid hydrolyzate contained streptolydin (which re-

ceived the special name roseonin), L-B-lysin, hexosamine (probably identical to D-gulosamine) and a compound (identical in its properties with N-guanyl-streptolydyl gulosaminide. Based on the analytic data and potentiometric titration this compound was erroneously assigned the doubled empirical formula $C_{24}H_{44}N_{10}O_{13}\cdot 6HCl = 2C_{12}H_{22}N_5O_{6-7}\cdot 3HCl$ (compare with the formula $C_{12}H_{23}N_5O_7\cdot 3HCl$) and the following structural formula:



Accordingly roseothricin A (which in all its properties is very similar to streptothricin) was ascribed the partial structure



which, we too believe to be erroneous.

It must be pointed out, the all arguments in favour of this structure, are in good accord with the formula, somewhat later proposed by American scientists for streptothricin.

In 1958, in a comparative chromatographic study of a number of streptothricin preparations it was shown that the main component of roseothricin A was identical in Rf with streptothricin, and the minor component with streptothricinic acid - the product of inactivation of streptothricin. In subsequent papers it was shown that the coincidence of the Rf values of compounds obtained from

different crude streptothricin preparations with that of streptothricin was not fortuitous. These preparations (for example p plecocidin I and antibiotic A 8235-I) were really identical with streptothricin.

Taking into consideration all these data and the close similarity of the properties and empirical formulas, it is reasonable to conclude that the main component of roseothricin A actually is streptothricin.

Up to the present time the streptothricins have been very little investigated in other aspects. Thus there are almost no data on their structure - activity relationships. ~~As the first material concerning this problem~~ allow me to show you the following table, which presents the activity of different streptothricins against a number of microorganisms. As one can see from the table, streptothricins with long peptide chain are more active then the first members of the series.

Table 6.

Antibacterial activity of streptothricins

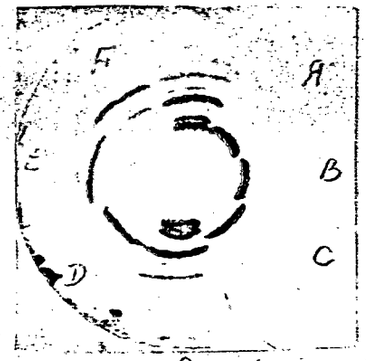
Microorganisms	Minimal inhibitory consentrations (γ/ml)					
	F	E	D	C	B	A
Staph.aureus 209P	3-6	0,3-0,5	0,05-0,1	0,05	$3\text{-}7.10^{-3}$	5.10^{-3}
Staph.aureus № 5	15	5	0,3-0,4	0,3	0,2	0,03
Sarcina lutea	5	5	0,05-0,1	0,07	0,05	0,05
Bacillus subtilis	9	2	0,4	0,2	0,03-0,07	0,1-0,15
Micobact.phlei	2	2	0,1	0,05	0,01	0,03-0,07
E.coli	37	18	9-18	9-18	9-18	6-7
Sacch.cerovisiae	50	18	2	1,5	0,5-07	0,1-05

There are practically no data on the mechanism of biosynthesis of streptothricins or on the influence of fermentation conditions on the formation of the different types of these compounds. Such investigations are now in progress in our laboratory.

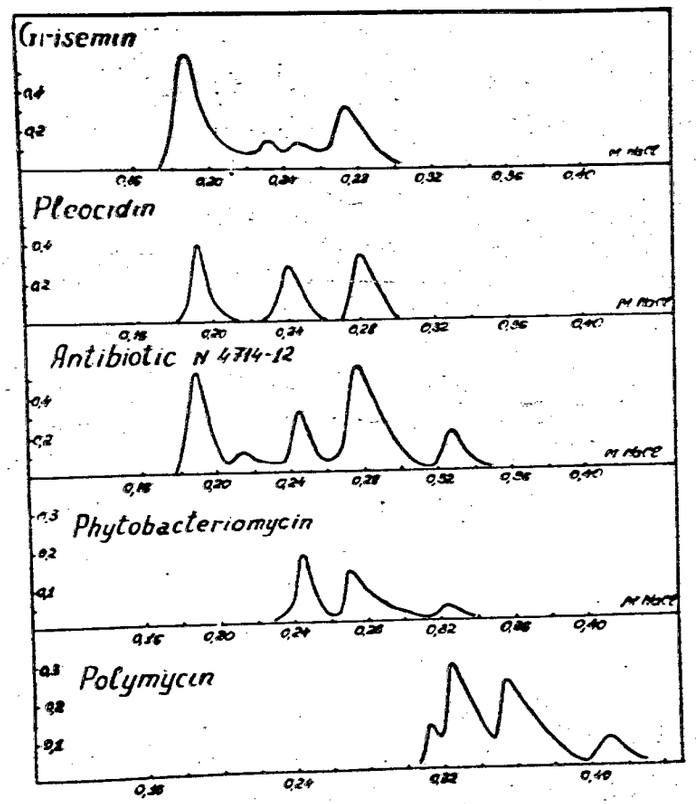
This then is the present state of our knowledge of this very peculiar group of antibiotics.

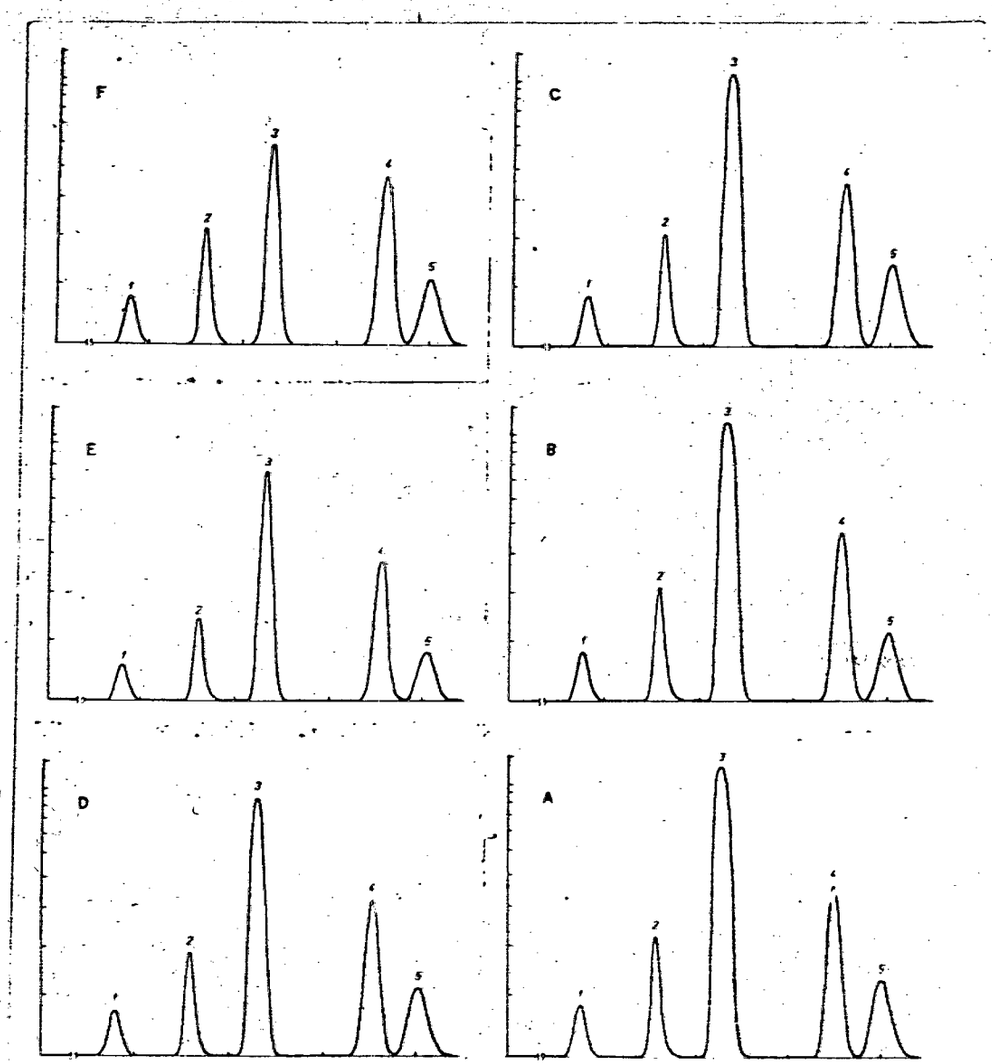
In connection with our studies we are very anxious to obtain different streptothricin preparations, especially those containing new or seemingly new components. We shall be very grateful to all those of our colleagues, who could furnish us with such samples for further investigation; and with this request allow me to finish.

Thank you for attention!

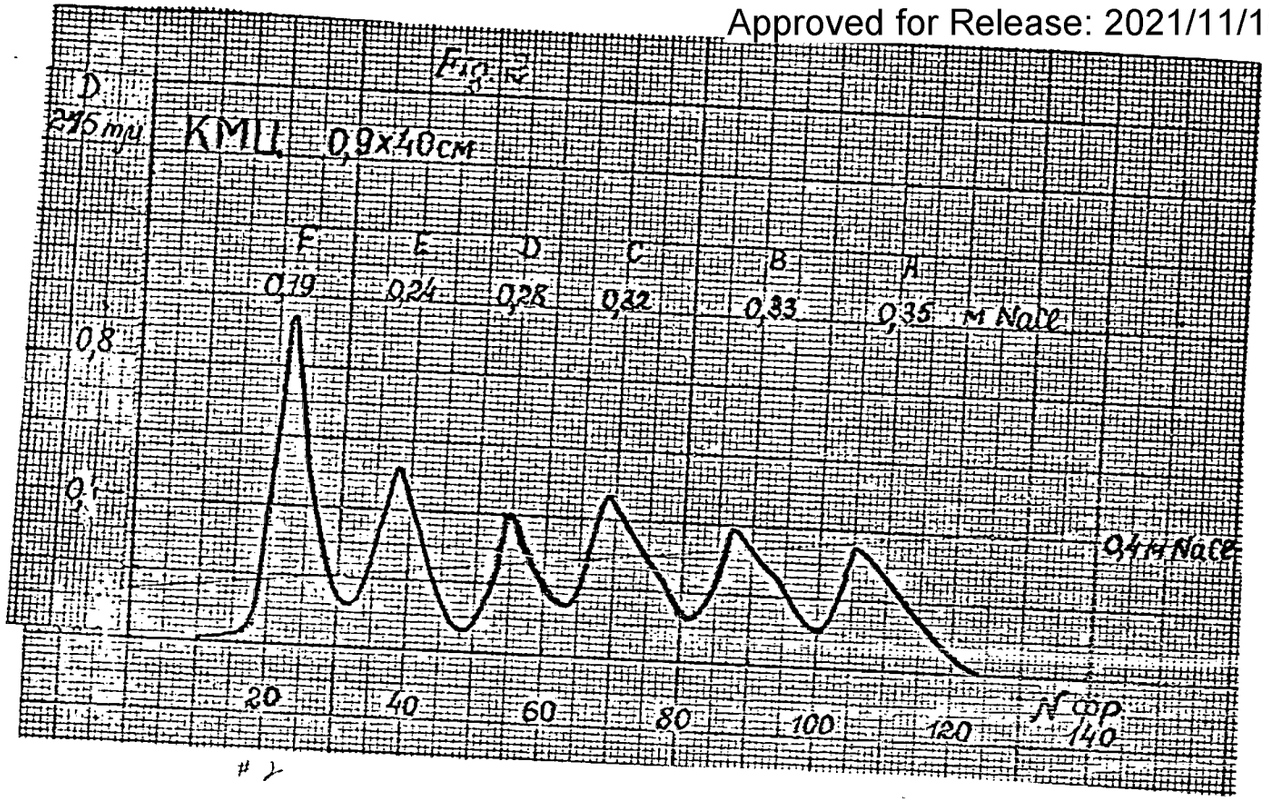


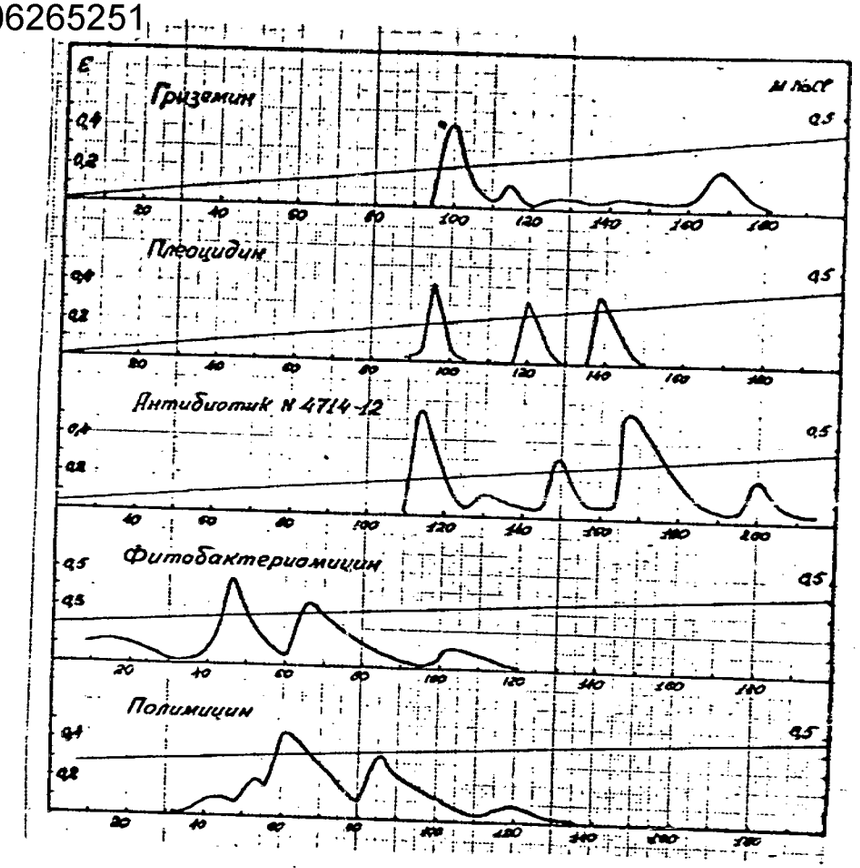
#8

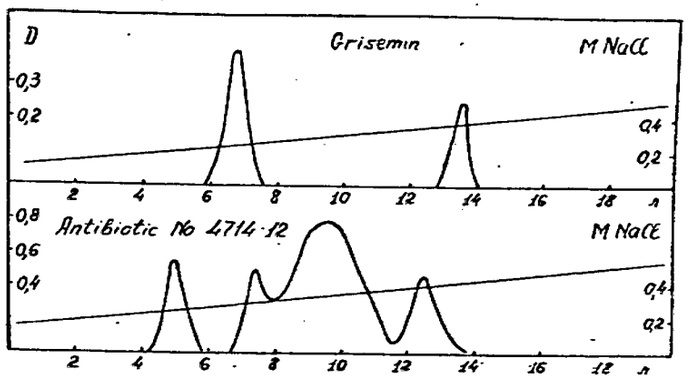


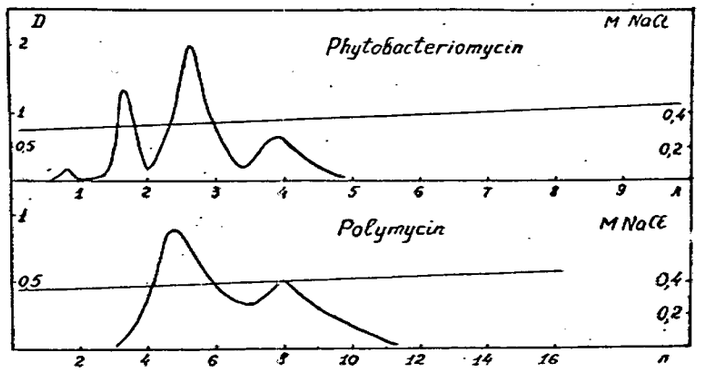


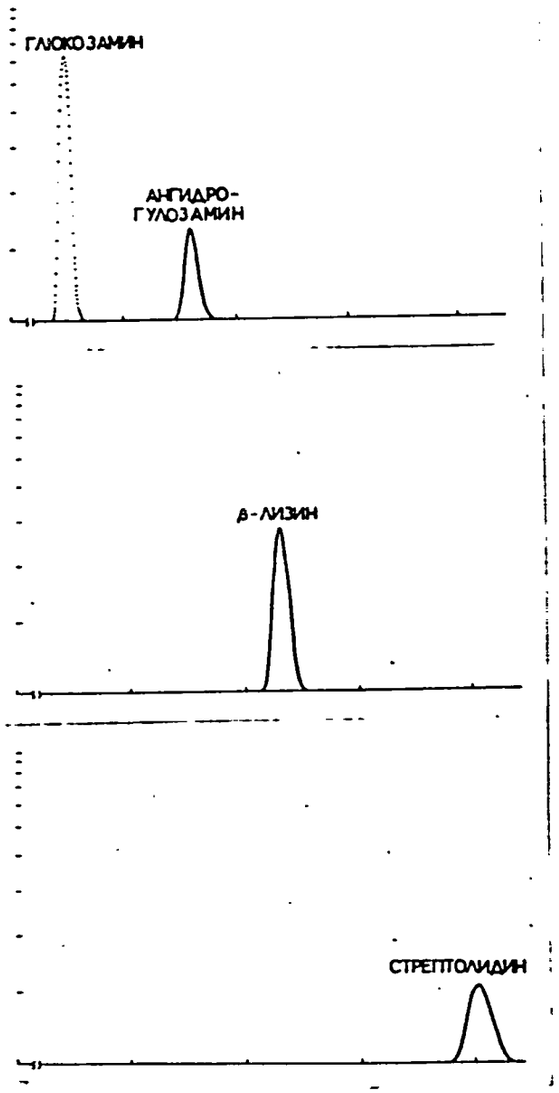
#7





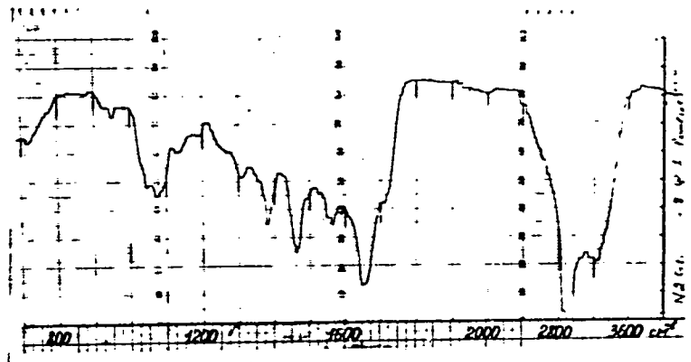




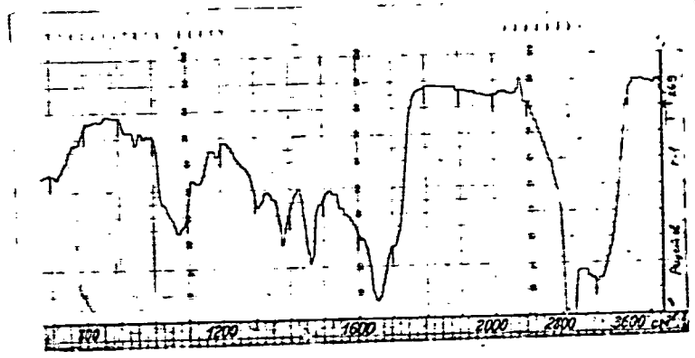


1

3



4



4