STUDIES ON STREPTOTHRICINS
VI. PREPARATION AND PROPERTIES OF INDIVIDUAL STREPTOTHRICINS

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The study of the streptothricins — a group of antibiotic bases of actinomycetous origin possessing a high and many-sided biological activity — has for a long time been somewhat spasmodic. One of the reasons for this phenomenon, in our opinion, is the ability of the producers of the streptothricins to form simultaneously from two to six antibiotics, the properties of which (insolubility in organic solvents, rapid inactivation in acid and alkaline media, absence of characteristic frequencies in the UV spectrum and of clear criteria of purity, and the like) seriously restrict the choice of methods of fractionation. The use for this purpose of partition chromatography on cellulose has proved to be so laborious that the production of the individual streptothricins has not infrequently overstepped the bounds of a laboratory experiment [1]. It is therefore characteristic that of the three compounds of this group the structure of which has been established [2, 3], two — streptolin and racemomycin-O — have not, apparently, been obtained in the pure state.

In the course of a systematic study of a new antibiotic of this group – polymycin – we carried out a chromatographic comparison of a number of streptothricin preparations, as a result of which it was established that components of all the mixtures studied belong to one of six types of substances generally characterized in the order of their R_f values by the indices A–F [4, 5]. Since the structure of representatives of the two most widely distributed types F (streptothricin) and D (streptolin) can be regarded as established, the greatest interest lies in the little-studied streptothricins of the series A, B, C, and E (in particular polymycins A and B). At the same time, it was very important for the classification of the streptothricins to compare the properties of the antibiotics possessing similar mobilities, since a comparison of the R_f values in only one system of solvents cannot be used as a decisive argument for their identity.

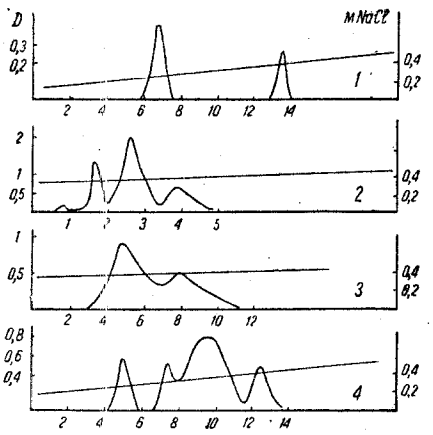

Fig. 1. Preparative fractionation of crude products with optimum loading of the column (4×90 cm): grisemin — 5 mg/ml (1), phytobacteriomycin — 2 mg/ml (2), polymycin and antibiotic No. 4714-12 — 1.5 mg/ml (3, 4).

In view of this, we set ourselves the aim of obtaining and characterizing a series of streptothricins consisting of components of preparations of polymycin, phytobacteriomycin, grisin (grisemin) and antibiotic No. 4714-12.

The isolation and subsequent purification of the individual antibiotics was carried out by a scheme that we have developed, the key stage of which is ion-exchange chromatography of preparations of the raw materials on carboxymethylcellulose, which has been briefly described in an earlier paper [5]:

Main stages of isolation and purification	Operations performed in each stage
1. Preliminary purification of the total preparation	a) Treatment with activated carbon b) Production of the picrate and its conversion into the hydrochloride
2. Fractionation of the total preparation	a) Ion-exchange chromatography on carboxymethylcellulose in an NaCl concentration gradient
3. Isolation of streptothricins from the eluates	a) Adsorption on Amberlite IRC-50 b) Desalting of the ion-exchanger c) Elution of the antibiotics
4. Final purification	a) Reprecipitation of the picrates and hydrochlorides b) Preparation of the oxalates and sulfates

The results of the preparative chromatography of the four preparations mentioned with optimum loading of the column are shown in Fig. 1. As the content of low-mobility components (C, B, A) in the preparations increases, satisfactory separation can be achieved only with a considerably lower loading of the column. It has been established that the limiting loads in the fractionation of the streptothricins, referred to the series D and F (grisemin), C and D (phytobacteriomycin), and A and B (polymycin) are, respectively, 10, 5, and 2.5 mg/ml.

The third stage of the process is extremely important, since the ratio by weight of the antibiotic and sodium chloride in the eluates is about 1:50. The concentration and desalting of the streptomycins has been achieved by adsorption on Amberlite IRC-50; however, as shown by analysis, the substances obtained at this stage still contained inorganic salts (10% of NaCl) and also traces of inactivation product, and therefore required further purification. In all, from

the four preparations mentioned, fourteen chromatographically distinct streptothricins were obtained and characterized in detail.

All the compounds belonging to the series A-E had a similar characteristic IR spectrum with clearly expressed amide bands: 3250, 1658, 1563, and 1310 cm^{-1}. The IR spectrum of the streptothricins of series F are characterized only by the absence of the second amide band in the 1563 cm^{-1} region (Fig. 2). The decrease in the chromatographic mobility of the streptothricins (from F to A) is accompanied by a smooth decrease in their $[\alpha]_D$ (Table 1).

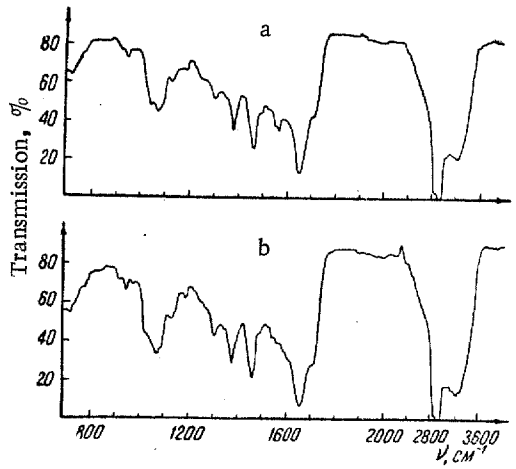

Fig. 2. IR spectra of the hydrochlorides of the streptothricins of series A-E (a) and series F (b)

One of the most important characteristics of compounds of the streptothricin type is the number of free amino groups, which determines their basicity. To determine the number of amino groups in the various types of streptothricins, we used a recently reported method based on the electrophoresis of the N-3,5-dinitro-1-sulfophenyl (DNSP) derivatives of various degrees of substitution [6]. The advantage of the DNSP derivatives over the dinitrophenyl (DNP) derivatives, consists of their high solubility in aqueous electrolytes, and also in the change in the total charge of the molecule as their degree of substitution increases right down to -2.

Since the dinitrosulfophenylation of the streptothricins in the presence of triethylamine is accompanied by their inactivation, leading to the formation of additional spots on the electrophoregram, we determined the number of amino groups in the six types of streptothricins and the products of their inactivation under the same conditions (Fig. 3). This comparison made it possible to leave the additional spots out of consideration in the interpretation of the electrophoregrams. The electrophoregrams of the DNSP-substituted streptothricins (Fig. 4) can be interpreted in the following way. As is well known, streptothricin, which has two amino groups, nevertheless adds three equivalents of acid, apparently because of the presence of a heterocyclic ring.

TABLE 1

Initial preparation	$[\alpha]_D$ of the hydrochlorides of streptothricins of the various types (in methanol, C 0.9-1.0)					
	A	*B*	*C*	*D*	*E*	*F*
Polymycin	—9.2	—9.6	—8.5			
Phytobacteriomycin			—12.8	—21.6		
Grisemin				—21.0	—34.1	—35.7
Antibiotic No. 4714-12			—12.2	—22.0	—36.0	—34.4
Streptothricin						—42.0
Streptolin				—22.0*		—51.0*

* Published data.

It follows from this that on electrophoresis in a strongly acid medium, its DNSP derivatives must have charges not of 0 and −2, but of +1 and −1, respectively. In actuality, on the electrophoregram these derivatives are well marked in the form of intensely colored spots symmetrically arranged with respect to the zero line.

As mentioned above, the minor spots (with charges of +2 and -2) belong to the DNSP derivatives of the inactivation product which, consequently, has one amino group more than the initial antibiotic. On the basis of these data and these taking into consideration the monotypic nature of the structure of streptothricin, streptolin and racemomycin-O, it is possible to interpret the electrophoregrams of the DNSP derivatives of the other antibiotics, in spite of the indefinite separation of the derivatives having a higher degree of substitution (Table 2).

The determination of the basicity of the streptothricins of the various types makes it possible to characterize the predominant components of some of the preparations in more detail. Using Amberlite IRA-400 in the appropriate form, the strongly hygroscopic amorphous hydrochlorides of these compounds were converted into the vitreous neutral sulfates and oxalates. Although they are somewhat less hygroscopic, these salts nevertheless strongly retain such polar

solvents as water and methanol, which, of course, influences the results of elementary analysis. Since these compounds slowly decompose on heating, their drying requires great care.

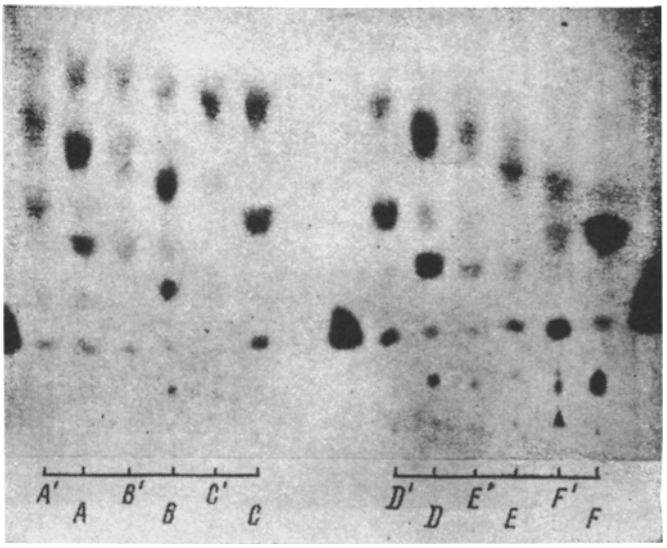

Fig. 3. Electrophoresis of DNSP derivatives of the six types of streptothricins and the products of their inactivation: A' – polymycin A, inactivated; A – polymycin A; B' – polymycin B, inactivated; B – polymycin B; C' – phytobacteriomycin C, inactivated; C – phytobacteriomycin C; D' – phytobacteriomycin D, inactivated; D – phytobacteriomycin D; E – grisemin E, inactivated; E – grisemin E; F' – grisemin F, inactivated; F – grisemin F.

In order to determine their equivalent weights, we used the oxidimetric titration of the oxalic acid previously precipitated from solutions of the oxalates in the form of calcium oxalate [7]. The results of titration and of elementary analysis, in association with the basicities of the streptothricins of the various types that have been found, permitted rough molecular weights and empirical formulae of the oxalates of the main components to be determined (Table 3).

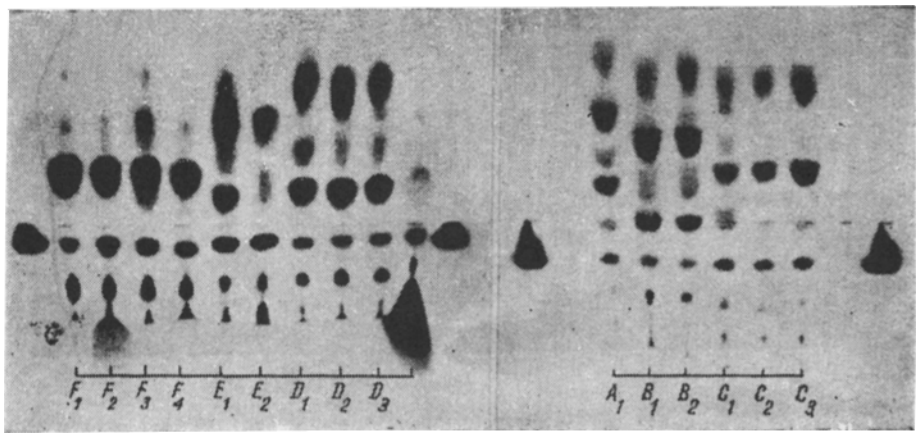

Fig. 4. Electrophoresis of the DNSP derivatives of 15 streptothricin antibiotics: F_1 – streptothricin; F_2 – grisemin F; F_3 – antibiotic No. 4714-12 F; F_4 – phytobacteriomycin F; E_1 – grisemin E; E_2 – antibiotic No. 4714-12 E; D_1 – grisemin D; D – phytobacteriomycin D; D_3 – antibiotic No. 4714-12 D; C_1 – phytobacteriomycin C; C_2 – antibiotic No. 4714-12 C; C_3 – polymycin C; B_1 – polymycin B; B_2 – phytobacteriomycin B; A_1 – polymycin A.

In conclusion, it must be recalled that the results of a study of the antibacterial activity of the compounds obtained confirm the assumption of the existence of six types of streptothricin antibiotics.

TABLE 2

Type of streptothricins	Charges and arrangement on the electrophoregram of the DNSP streptothricins	No. of amino groups	Basicity of the 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

TABLE 3

Antibiotics	Mp with decomp., °C		Mol. wt. of the oxalates		Empirical formula of the oxalates
	Oxalates	Sulfates	Found	Calculated	
Phytobacteriomycin F	178.5—180				
Antibiotic No. 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 B	184—186	214—215	1040	1001	$C_{31}H_{58}N_{12}O_{10} \cdot 2{,}5C_2H_2O_4 \cdot H_2O$
Grisemin	184—186		1015	1001	$C_{31}H_{58}N_{12}O_{10} \cdot 2.5C_2H_2O_4 \cdot H_2O$
Phytobacteriomycin C	183.5—185	215—217	1176	1174	$C_{37}H_{70}N_{14}O_{11} \cdot 3C_2H_2O_4 \cdot H_2O$
Polymycin B	181—183	216—217	1393	1347	$C_{43}H_{82}N_{16}O_{12} \cdot 3.5C_2H_2O_4 \cdot H_2O$
Polymycin A	181—183	>216.5	1496	1520	$C_{49}H_{94}N_{18}O_{13} \cdot 4C_2H_2O_4 \cdot H_2O$

Experimental

In view of the similarity of the properties of the streptothricins, we give below a description of the standard operations on the basis of individual examples, with suitable notes in those cases where, because of the properties of the substances studied, it was necessary to introduce certain changes into the method.

Preliminary purification of the total preparation. After their isolation from the culture liquid, the streptothricin preparations generally contain about 20% of inorganic salts, together with colored impurities which also make preliminary purification necessary.

A solution of 12 g of crude phytobacteriomycin sulfate in 20 ml of water (generally brown in color) was filtered under pressure through a column (1.5× 5 cm) filled with a mixture of BAU and Celite 545 (a mixture of finely ground carbon of grade BAU partially inactivated with stearic acid and Celite 545 in a ratio of 1:1 was used to eliminate the colored impurities), the slightly colored eluate was concentrated to 20 ml, and the resulting solution was poured into 200 ml of a hot aqueous solution containing 16 g of picric acid and was left in the refrigerator overnight. After being washed with water (two 20-ml portions), the oily picrate was dissolved in 80-90 ml of aqueous acetone (3-5% of water); the resulting solution, after separation from the insoluble residue, was diluted with 50 ml of absolute ethanol, and, after cooling, the hydrochlorides of the streptothricins were precipitated by the addition of 15 ml conc. HCl.

After washing with acetone (two 20-ml portions), the oily hydrochloride was dissolved in the minimum volume of dry methanol (about 50 ml) and the slightly yellowish solution was passed through a small layer (2 ×5 cm) of finely ground (400-600 mesh) Amberlite IRA-400 ion-exchanger in the Cl form in order to eliminate traces of picric acid. The filtrate was evaporated to dryness in a rotary evaporator at a temperature not exceeding 35°; the oily residue was dissolved in dry methanol, a few milliliters of ethanol were added until slight turbidity appeared, and the resulting suspension was slowly evaporated to dryness with the addition of absolute ethanol from time to time. The amorphous highly hygroscopic hydrochlorides obtained in this way were stored in a vacuum desiccator in the presence of P_2O_5. The yield in this stage depends on the purity of the initial preparation and amounts to 70-80%.

Chromatography of the purified preparations in the butanol-pyridine-acetic acid-water (15:10:3:12) system showed that the samples were free from ninhydrin-positive impurities and contained only antibiotics of the streptothricin group.

Ion-exchange chromatography of the streptothricins of the preparations on carboxymethylcellulose (CMC) (Table 4). The streptothricin preparations were fractionated on a 4 × 90 cm column (volume 1 liter; adsorbent — CMC in the Na form with a capacity of 0.55 meq/g from the firm of Serva Entwicklungslabor (Heidelberg); some of the experiments were carried out on CMC obtained under laboratory conditions by a recognized method [5]) at the rate of 100 ml/hour with 200-250-ml fractions over 100-150 hours. Qualitative analysis of the fractions was carried out by means of the reaction with sodium phosphotungstate and quantitative analysis by measuring the optical density on a SF-4 spectrometer at 215 mμ.

The polymycin preparations were also fractionated on a 6 × 90 cm column (volume about 2 liters) at the rate of 200-250 ml/hr. The volume of the fractions was 250-300 ml, the concentration of the solutions in the mixer was 12 liters of 0.35 M NaCl, while the reservoir contained 12 liters of 0.45 M Na Cl. The amount of the preparation was 4–13 g.

TABLE 4

Characteristics	Grisemin	Antibiotic No. 4714-12	Phytobacteriomycin	Polymycin
Amount of preparation, g				
optimum	3—5	1.4	2	1.5
maximum *	10	1.4	5	2.5
Volume (liters) and concentration M of the NaCl solutions				
in the mixer	10—0.1	10—0.15	10—0.3	8—0.35
in the reservoir	10—0.5	10—0.55	10—0.6	8—0.45
Yield of the individual components, ** %	*F* 45 *E****55 *D* 40	*F* 20 *E* 15 *D* 50 *C* 15	*F* 5 *D* 60 *C* 30 *B* 5	*C****5 *B* 55 *A* 40

* When the initial preparations were insufficiently pure, chromatography was carried out twice, the predominant components being subjected to a preliminary separation with maximum loading of the column.

** The yields were actually somewhat less because the preparations obtained contained a certain amount of inorganic salts and inactivation products.

*** It was possible to isolate the components only from certain preparations of grisemin, phytobacteriomycin, and polymycin.

Isolation of the streptothricins from the eluates. The fractionation gave the individual components distributed in 2-5 liters of 0.2-0.3 M NaCl, the ratio by weight of the antibiotics and sodium chloride in the solution amounting to about 1:50 . In order to concentrate and desalt the compounds, we developed a method which enabled us to overcome a whole series of difficulties connected with the properties of the streptothricins.

The fractions containing polymycin B were combined (the total volume amounted to about 3 liters, or, when working with a 6 × 90 cm column, 5 liters) and were diluted two-fold with water; the resulting solution was passed through a small column (1.5 × 4 cm) containing the cation-exchanger Amberlite IRC-50 in the Na form (particle dimensions 80-100 μ) at the rate of about 500 ml/hr. As the Na ion was displaced by the antibiotic, the pink coloration of the cation-exchanger turned to pure white, and the saturation boundary was readily determined by visual inspection. It is advantageous to carry out the adsorption until the cation-exchanger is completely saturated with the substance. After the end of the adsorption, the residual Na ion was displaced with 0.01-0.02 N acetic acid at the rate of about 200 ml/hr until polymycin B appeared in the eluate, after which the antibiotic was desorbed with 0.1 N HCl at the rate of 50-100 ml/hr, the completeness of the elution being checked by the reaction with sodium phosphotungstate. The neutral and acid eluates, amounting to about 50 ml each, were collected separately. The acid eluate was neutralized with the anion-exchanger Amberlite IR-45 in the OH form to pH 5-6, combined with the neutral eluate, and evaporated to dryness at a temperature not exceeding 40°. The glassy hydrochloride was dissolved in dry methanol and the solution was evaporated to dryness in the presence of absolute ethanol, as described above. The amorphous hygroscopic hydrochlorides were stored in a vacuum desiccator over P_2O_5.

Purification of the hydrochlorides of the streptothricins obtained after fractionation. Purification of the hydrochloride of polymycin B. A solution of 600 mg of the hydrochloride of the antibiotic in 5 ml of water was added to a hot solution of 1 g of picric acid in 15-20 ml of water and the mixture was left overnight in the refrigerator. The oily picrate was dissolved in 5-6 ml of aqueous acetone (about 10% of water), the insoluble residue was separated by centrifuging, and the picrate was precipitated with 25-30 ml of absolute ether. The oily residue was again dissolved in

5-6 ml of aqueous acetone, and the solution was diluted two-fold with aqueous methanol (about 3% of water) and passed through a column (1.2 × 4 cm) consisting of three layers - about 2 ml of a mixture of BAU and Celite 545, 2 ml of Celite 545, and about 4 ml of carefully washed, finely ground (400-600 mesh) Amberlite IRA-400 ion-exchanger in the Cl form (layers enumerated from the bottom upwards). The hydrochloride of polymycin B was eluted with aqueous methanol, the eluate evaporated to dryness, the residue again dissolved in dry methanol, and the resulting solution was evaporated to dryness in the presence of ethanol as described above. Yield 520 mg.

Purification of the hydrochloride of polymycin A. A solution of 1.5 g of the hydrochloride of the antibiotic in 5 ml of water was mixed with a hot saturated solution of 2 g of picric acid in water and the mixture was left in the refrigerator overnight. The oily picrate was dissolved in 10 ml of aqueous acetone (about 5% of water), the insoluble residue was separated off, and the picrate was precipitated with 50 ml of absolute ether. The precipitate was again dissolved in aqueous acetone (about 20% of water), and a small amount of aqueous methanol (about 20% of water) was added until the appearance of slight turbidity. The solution was passed through a column containing three layers - a mixture of BAU and Celite 545, Celite 545, and 10 ml of finely ground (400-600 mesh) Amberlite IRA-400 ion-exchanger in the Cl form. The methanolic eluate was evaporated to dryness, and the polymycin A hydrochloride was dried as described above. Yield 1.3 g.

It was possible to remove the contaminating inactivation products by the fractional reprecipitation of the hydrochlorides from methanolic solution with absolute ethanol by the following procedure. A solution of 500 mg of desalted polymycin B hydrochloride in 4-5 ml of dry methanol was separated from the insoluble residue and 3-4 ml of a mixture of methanol and absolute ethanol (1:1) was added. The precipitate (about 1/3 of the initial amount of substance) was separated off and the alcoholic solution was evaporated to dryness as described above.

Identification of the compounds obtained. The IR spectra of the streptothricin hydrochlorides were recorded on a UR-10 instrument in a liquid paraffin mull, and the specific rotations at various wavelengths on a Russian-made spectropolarimeter.

TABLE 5

Antibiotic	Element composition, %			
	C	H	N	S
Phytobacteriomycin D oxalate	43.20	6.32	16.84	
	43.29	6.44	16.94	
Grisemin D oxalate	43.02	6.36	17.28	
	43.12	6.49	16.99	
Antibiotic No. 4714-12 D oxalate	42.88	6.52	16.22	
	43.17	6.42	16.31	
Phytobacteriomycin C oxalate	44.30	6.47	16.96	
	44.36	6.49	16.99	
Phytobacteriomycin C sulfate	37.10	6.76	16.08	8.00
	36.94	6.82	16.04	7.93
Polymycin B oxalate	44.43	6.84	16.28	
	44.38	6.96	16.11	
Polymycin B sulfate	37.57	7.02	16.27	7.95
	37.44	6.91	16.27	7.91
Polymycin A oxalate	44.67	6.72	16.46	
	44.67	7.04	16.27	
Polymycin A sulfate	38.02	7.18	16.38	8.18
	37.85	7.02	16.51	8.06

Determination of the number of free amino groups in the six types of streptothricin and the products of their inactivation. The inactivation products of representatives of the six types of streptothricins - grisemins F and E, phytobacteriomycins D and C, and polymycins B and A — were obtained under the following conditions. A solution of 50 mg of the antibiotic in 3-4 ml of 2.5 N HCl was kept at 20° for 10 days, after which the solution was evaporated to dryness at a temperature not exceeding 35°. The inactivation product was dried with dry methanol and absolute ethanol as described above, and the residual HCl was eliminated by storing the samples in a vacuum desiccator over alkali. The homogeneity of the inactivation products was confirmed by paper chromatography in the butanol-pyridine-acetic acid-water (15:10:3:12) system. Potassium 4-chloro-3,5-dinitrobenzenesulfonate (CDNBS) was synthesized by a published method [6]. It had been shown by preliminary experiments that the majority of the DNSP derivatives of various degrees of substitution were formed under the following reaction conditions. To 3 mg of the hydrochloride of the antibiotic in 0.1-0.2 ml of water were added 0.2 ml of a 5% solution of CDNBS in 50% aqueous acetone (about 10 mg or 0.03 meq of CDNBS) and 0.1 ml of 10% aqueous triethylamine. The reaction mixture was thermostated at 50° for 1.5 hr, after which part of the solution was deposited on the electrophoregram in a current of hot air.

Electrophoresis was carried out on paper from the Goznak mill (sheet dimensions 50 × 26 cm) in the apparatus for vertical electrophoresis described by Mikes [8]. The electrolyte was 85% formic acid-acetic acid-water

(30:30:40 or 5:30:65), the voltage 400 v, the current strength 0.2-0.3 ma/cm, and the time 7-8 hr. To determine the position of the zero line, DNP–glycine was placed at the starting line. A blank sample - a mixture of CDNBS and triethylamine stored under the same conditions as the sample with the antibiotics - was also deposited on the electrophoregram. The method of calculating the number of amino groups in the streptothricins has been discussed above.

TABLE 6

Type of Streptothricins	Calculated empirical formula	Element composition, %			
		C	H	N	S
D oxalate	$C_{31}H_{58}N_{12}O_{10}\cdot 2.5C_2H_2O_4\cdot H_2O$	43.10	6.50	16.80	—
C oxalate	$C_{37}H_{70}N_{14}O_{11}\cdot 3C_2H_2O_4\cdot H_2O$	43.90	6.64	16.70	—
C sulfate	$C_{37}H_{70}N_{14}O_{11}\cdot 3H_2SO_4\cdot H_2O$	37.00	6.50	16.35	8.00
B oxalate	$C_{43}H_{82}N_{16}O_{12}\cdot 3.5C_2H_2O_4\cdot H_2O$	44.60	6.76	16.60	—
B sulfate	$C_{43}H_{82}N_{16}O_{12}\cdot 3.5H_2SO_4\cdot H_2O$	37.55	6.62	16.30	8.15
A oxalate	$C_{49}H_{94}N_{18}O_{13}\cdot 4C_2H_2O_4\cdot H_2O$	45.00	6.84	16.70	—
A sulfate	$C_{49}H_{94}N_{18}O_{13}\cdot 4H_2SO_4\cdot H_2O$	37.90	6.70	16.35	8.26

Preparation of the oxalates and sulfates of the streptothricins. A solution in 4-5 ml of water of 100-150 mg of polymycin B hydrochloride, desalted and purified from inactivation product, was passed through a column (0.6×4 cm) containing carefully washed, finely-ground (400-600 mesh) Amberlite IRA-400 anion-exchanger in the oxalate or the sulfate form. The completeness of the elution was checked by the reaction with sodium phosphotungstate. The eluate was evaporated to dryness and the vitreous residue was dried for 1 hour in a rotary evaporator at 30-35°. The dried vitreous mass was treated with 5-10 ml of absolute ethanol and and left overnight. After 12 hr, the ethanol was distilled off to dryness, and the neutral powdery salts of the antibiotics were dried for 7-10 days in a high vacuum over P_2O_5, the desiccant being replaced by a fresh sample every three days. The decomposition temperatures of the oxalates and sulfates of a series of streptothricins are given in Table 3 and the found and calculated element compositions of the salts of a series of streptothricins are given in Tables 5 and 6.

TABLE 7

Substance	Sample, mg	0.1 N $KMnO_4$, ml	Oxalic acid mg	Equivalent weight	
				Found	Calculated for formula in Table 6
Lysine monooxalate	18.25	0.96	4.32	145	146
	22.35	1.18	5.32	144.5	
Grisemin F	15.20	0.70	3.15	172	173
	15.90	0.73	3.28	173	
Grisin D	12.80	0.64	2.88	155	155
	13.60	0.66	2.97	161	
Phytobacteriomycin D	13.3	0.64	2.88	163	155
Phytobacteriomycin C	13.6	0.70	3.15	149	151
	14.3	0.72	3.24	153	
Polymycin B	12.8	0.64	2.88	155	148
	14.9	0.75	3.37	154	
Polymycin A	13.0	0.69	3.13	142	145

Determination of the equivalent weights of the streptothricins by the oxidimetric titration of the oxalates. An accurately weighed sample of the streptothricin oxalate (about 12-15 mg) in 3-4 ml of water was placed in a centrifuge tube, 4 ml of 0.5% $CaCl_2$ solution was added, and the mixture was heated for 1 hour in a boiling water bath. The clear solution was decanted off, and the precipitate of Ca oxalate was washed with 4 ml of cooled water and dissolved in 5-6 ml of 2 N H_2SO_4. The resulting solution was heated to 80° and titrated with 0.1 N $KMnO_4$ from a microburette with constant stirring until the appearance of a pink coloration which did not disappear for 1 min. One ml of 0.1 N $KMnO_4$ corresponds to 0.004502 g of oxalic acid. Table 7 gives the results of the titration of lysine monooxalate as a model and of the neutral oxalates of a series of streptothricins.

SUMMARY

1. A general scheme for the preparative preparation of the individual antibiotics of the streptothricin group has been worked out. Fourteen individual antibiotics belonging to the six types of streptothricins have been isolated and characterized. It has been shown that three samples of type D, as well as two antibiotics of type C, are identical with one another.

2. The empirical formulae of the following new streptothricins have been established: phytobacteriomycin C (streptothricin C) and polymycins A and B (streptothricins A and B).

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