Reaction of Phenanthraquinone with Benzaldehyde, Ammonium Hydroxide and Sodium Hydroxide.—The reactants were mixed in the same proportions as used in the above experiment (omitting the cupric acetate) and heated for two hours. Most of the product separated on cooling and the remainder was obtained by concentrating the filtrate. The 2-phenylphenanthrimidazole and 2-phenylphenanthroxazole were separated by the cupric acetate method; yield of imidazole was 70–73%, m. p. 312–313°; yield of oxazole 3–7%, m. p. 204–205°.

Reaction of Phenanthraquinone with Benzaldehyde and Ammonia in the Presence of Piperidine.—Phenanthraquinone (2.08 g., 0.01 mole) was suspended in 50 cc. of alcohol and 100 cc. of 28% aqueous ammonia added. A solution of 1.3 g. (0.012 mole) of benzaldehyde and 1.4 g. (0.016 mole) of piperidine in 10 cc. of alcohol was then added and the mixture heated on a steam-bath for one hour. The mixture was cooled and the solid removed by filtration. The crude product was treated as described previously. The sole detectable product was 2-phenylphenanthroxazole, yield 68%, m. p. 204.5–205°.

Reaction of Phenanthraquinone with Salicylaldehyde, Cupric Acetate, Ammonium Hydroxide and Sodium Hydroxide.—See the corresponding reaction with benzaldehyde for the procedure followed. The precipitate which formed during the course of the reaction was a mixture of the copper salts of the corresponding imidazole and oxazole. This behavior was not surprising in view of the phenolic nature of the hydroxyl group present. The copper salts were suspended in hot alcohol and decomposed with hydrogen sulfide. The filtrate from the copper sulfide was concentrated to obtain the mixture of oxazole and imidazole. Several recrystallizations from aqueous pyridine were required to separate the more soluble oxazole from the imidazole. Yield of 2-(2'-hydroxyphenyl)-phenanthroxazole was 9.5%, m. p. 246–247°[10]; yield of 2-(2'-hydroxyphenyl)-phenanthrimidazole, 54%, m. p. 287–288°. Mixed melting points with authentic samples showed no depression.

Reaction of Phenanthraquinone with *m*-Nitrobenzaldehyde, Cupric Acetate, Ammonium Hydroxide and Sodium Hydroxide.—The procedure for the corresponding reaction with benzaldehyde was used in this case without modification. The crude 2-(3'-nitrophenyl)-phenanthrimidazole was recrystallized from pyridine and water, yield 77.8%, m. p. 271–272° [11] No oxazole was isolated from this reaction.

Summary

1. A method for the preparation of phenanthrimidazoles from phenanthraquinone, aromatic aldehydes and ammonium hydroxide in the presence of sodium hydroxide has been developed. The probable role of the sodium hydroxide has been discussed.

2. A method for the separation of most phenanthrimidazoles from phenanthroxazoles through the use of cupric acetate has been described.

(10) Stein and Day, THIS JOURNAL, **64**, 2567 (1942).

(11) Steck and Day, *ibid.*, **65**, 452 (1943).

PHILADELPHIA, PENN. RECEIVED AUGUST 31, 1945

[CONTRIBUTION FROM THE MERCK RESEARCH LABORATORIES, MERCK & CO., INC.]

Streptomyces Antibiotics. VI. Isolation of Streptothricin

BY ROBERT L. PECK, ALPHONSE WALTI,[1] ROBERT P. GRABER, EDWIN FLYNN, CHARLES E. HOFFHINE, JR., VINCENT ALLFREY[2] AND KARL FOLKERS

Methods have been found for the purification and isolation of streptothricin from the culture broths of *Streptomyces lavendulae*.

Crystalline salts of streptothricin and streptomycin have been described.[3] The preparation of streptothricin helianthate[3] and other procedures for the purification of streptothricin are described herein.

Streptothricin concentrates were first prepared by Waksman and Woodruff[4] from the culture broths of *Streptomyces lavendulae*. After treatment with Norite-A, the adsorbate was eluted with dilute acid and the eluate was neutralized and evaporated to a concentrate which was used for biological studies. The adsorbate was also eluted with acidified alcohol, and the eluate was neutralized and treated with ether to give a precipitate which was used for biological tests.[5]

Fried and Wintersteiner[6] described the crystalline reineckate of streptothricin which was stated to have been obtained by the following sequence of steps: charcoal adsorption, elution with mineral acid, precipitation with phosphotungstic acid, conversion of regenerated bases to crude picrate, chromatography of picrate, reineckate.

Although our methods for obtaining streptothricin had certain steps which were modifications of those described above, other steps were different. Thus, the following sequence of steps has been used satisfactorily: charcoal adsorption, elution with formic acid solutions, precipitation with picric acid and direct conversion to hydrochlorides, chromatography of hydrochlorides, helianthate.

After a study of elution procedures, it was found that the streptothricin could be eluted satisfactorily from the charcoal adsorbate by a solution of formic acid in methyl alcohol–water. The eluate was then partially concentrated *in vacuo*. Addition of acetone caused the formation of a precipitate which showed 40-80 units/mg. activity. The recovery of activity as the formate from the broth was 30–60%.

Further purification was effected by treating the crude formate or hydrochloride with picric acid in water. The somewhat selective separation of streptothricin picrate resulted in a con-

(1) Biochemical Division, Interchemical Corp., Union, New Jersey.

(2) U. S. Army.

(3) Kuehl, Peck, Walti and Folkers, *Science*, **102**, 34 (1945).

(4) Waksman and Woodruff, *Proc. Soc. Exp. Biol. Med.*, **49**, 207 (1942).

(5) Waksman, *J. Bact.*, **46**, 299 (1943).

(6) Fried and Wintersteiner, *Science*, **101**, 613 (1945).

siderable increase in the activity of the concentrate. The crude picrate was generally treated directly with hydrochloric acid, and a concentrate of streptothricin hydrochloride was isolated which showed 125–150 units/mg. activity. The recovery of the hydrochloride from the formate was 40–80%.

Concentrates of streptothricin hydrochloride obtained from the formate were chromatographed over aluminum oxide or a mixture of Darco G-60 and paper pulp. These procedures gave fractions which had significantly higher activities, *i. e.*, 500–730 units/mg., and that using aluminum oxide was a very useful step.

Methyl orange and preparations of streptothricin hydrochloride in methyl alcohol–water yielded crystalline streptothricin helianthate. After the helianthate had been recrystallized to constant properties, the regenerated streptothricin hydrochloride had an activity of 830 units/mg. and a specific rotation $[\alpha]^{25}D$ −51.3° (*c*, 1.4 in water).

Concentrates of streptothricin formate were also treated with phosphotungstic acid and the precipitated phosphotungstates were converted to concentrates of streptothricin sulfate which showed an average activity of 220–330 units/mg.

Several samples of crude streptothricin picrolonate were obtained which could be crystallized from methyl alcohol–water. Flavianic acid showed a definite specificity as a precipitant when it was used on solutions of the hydrochloride of 400–500 units/mg. activity. The flavianates were converted to hydrochlorides with 550–700 units/mg. activity.

It was found during the early purification studies that crystalline reineckates of streptothricin and streptomycin could be prepared. Streptothricin reineckate, of 300 units/mg. activity, was obtained. It was converted to either the hydrochloride or the hydrobromide by using the corresponding pyridine salt. Streptomycin reineckate was obtained which had an activity of 375 units/mg. Purification of the streptomycin reineckate, which was obtained from hydrochloride of low potency, was effected by partition of the reineckate between water and an organic solvent such as ethyl acetate or isoamyl alcohol. Streptomycin hydrochloride with 170 units/mg. activity was converted to the reineckate, and the wet filter-cake was dissolved in ethyl acetate, and after extracting the solution three times with water, the reineckate was recovered and converted to the hydrochloride. The product had 380 units/mg. activity which represented 65% recovery. Although purification was achieved with these reineckates, the difficulty in converting them to "ash-free" salts was a disadvantage. Reinecke's acid did not seem to be a particularly specific precipitant for concentrates of low potency.

Experimental

B. subtilis was used as the test organism in a cup assay method to determine antibiotic activity. We are indebted to Dr. H. B. Woodruff and Mr. D. Hendlin of the Microbiological Department for these assays.

Isolation of Crude Concentrates of Streptothricin Formate from Broth.—The following procedure has been found to give amorphous concentrates of streptothricin formate having an activity of 40–80 units/mg., in 30–60% yields, when applied to streptothricin broth of 40–80 units/cc. activity.

The broth was filtered through a thin pad of filter-cel to remove suspended solids, and the filtrate treated with 7.5 g. of Norite per liter. This suspension was stirred for one hour at room temperature and finally filtered. The adsorbate was then washed by stirring with 1 liter of ethyl alcohol per 100 g. of Norite for three-quarters of an hour. The Norite was removed by filtration and dried *in vacuo* at room temperature.

The streptothricin was eluted with 0.8 *N* formic acid in 1:1 methyl alcohol–water. This was accomplished by suspending the Norite in a volume of eluate equivalent to 15% of the volume of broth treated. After stirring four hours at 45°, the Norite was removed by filtration and the filtrate concentrated *in vacuo* to 0.01 of its original volume. Five volumes of methyl alcohol was added followed by four times the total volume of acetone. The resulting precipitate was removed by centrifuging, washed with ether, and dried *in vacuo*. The product varied in color from a light tan to a brown.

The results of three experiments using the above-described general procedure are summarized in Table I.

TABLE I

CONCENTRATES OF STREPTOTHRICIN FORMATE

Broth filtrate		Product		
Volume liters	Units/cc.	Wt., g.	Units/mg.	% Activity recovered
3.4	80	0.96	82	28
26	60	9.00	65	37
5	45	2.05	50	45

Purification of Streptothricin by Precipitation with Picric Acid.—It was found that a yellow gummy precipitate was obtained when aqueous solutions of streptothricin were treated with picric acid. The precipitate could be purified by dissolving it in methanol and adding ether to precipitate the crude streptothricin picrate as a yellow powder. The crude picrate was soluble in methanol and for conversion to the hydrochloride, a methanol solution of the picrate was acidified with hydrochloric acid and poured into ether causing precipitation of the hydrochloride. The recovery of active material in this step was practically quantitative. Tables II and III contain data on yields of the picrate and hydrochloride. The isolation of the picrate was not necessary since it could be converted directly to the hydrochloride. The following experiment illustrates this modification. Two hundred and forty-six grams of crude streptothricin hydrochloride, which showed 20 units/mg. activity, and 288 g. of picric acid was added to 7.1 liters of water and the mixture was stirred and heated until all solid dissolved. The solution was cooled to about 5° and allowed to stand for several hours. The sludge of streptothricin picrate and free picric acid was separated from the supernatant solution by decantation and dissolved in warm methanol containing an excess of 2.5 *N* hydrochloric acid. The hydrochloride was precipitated by pouring the solution into 10 volumes of ether. The precipitate was dissolved in methanol and reprecipitated with ether. After drying *in vacuo*, the streptothricin hydrochloride weighed 24 g. and showed 122 units/mg. activity.

Chromatographic Purification of Streptothricin with Aluminum Oxide.—Acid-washed aluminum oxide was used in a ratio of 10–30 g. of adsorbent to 1 g. of streptothricin hydrochloride concentrate. The columns were filled with

TABLE II

CONCENTRATES OF STREPTOTHRICIN PICRATE

Streptothricin hydrochloride used		Yield of streptothricin picrate		
Wt., g.	Activity, units/mg.	Wt., g.	Activity, units/mg.	% Activity recovered
5.0	13	1.70	14	38
5.0	18	2.31	21	54
5.0	18	2.28	20	50
46.0	21	9.00	63	59
2.0	46	2.66	28	77
121.0	18	13.0[a]	110[a]	66[a]
		2.8	133	17
71.0	25	11.1	60	37
182.5	12	19.0	74	64

[a] Two crops were obtained.

TABLE III

CONVERSION OF STREPTOTHRICIN PICRATE TO THE HYDROCHLORIDE

Streptothricin picrate used		Yield of streptothricin hydrochloride	
Wt., g.	Activity, units/mg.	Wt., g.	Activity, units/mg.
6.0	63	3.02	126
2.0	70	0.99	145

the adsorbent and methanol, and the solvent was allowed to drain until a layer of solvent above the adsorbent was approximately 1–2 mm. deep. The crude streptothricin hydrochloride was dissolved in methanol at a concentration of 10–50% and the solution was allowed to flow through the column either by gravity or under pressure of 10–20 mm. of mercury. When the solution was all in the adsorbent layer, methanol was added and allowed to percolate either by gravity or under pressure. The eluates were tested for the presence of solids by the addition of a few drops of the eluate to 5 ml. of acetone or ether. When precipitation of solids appeared in the test, the collection of fractions was begun. The eluates were concentrated to about one-tenth of the original volume and poured into acetone or ether or a mixture of both to precipitate the streptothricin hydrochloride products which were then collected by filtration or centrifugation and dried *in vacuo*. Table IV contains data on two typical experiments.

TABLE IV

CHROMATOGRAPHIC PURIFICATION OF STREPTOTHRICIN WITH ALUMINUM OXIDE

Concentrate used			Eluates		Product		
Wt., g.	Activity units/mg.	Adsorbent wt., g.	Number	Volume, ml.	Wt., g.	Activity units/mg.	% Activity recovered
10.0	49	160	1–2	148[a]	2.30	20	4
			3	30	0.76	64	10
			4–10	402[a]	2.48	101–123[b]	57
			11–14	452[a]	0.55	70–92[b]	9
							—
							80
107	450	1000	1	1000	43.4	688	62
			2	1000	18.9	648	25
							—
							87

[a] Combined volumes. [b] Highest and lowest average values for the eluates in the group.

Chromatographic Purification of Streptothricin with Darco G-60.—The columns were packed with dry mixtures of Darco G-60 and filter paper pulp. The solvent was introduced under pressure and the columns were ready to use when a layer of 1–2 mm. of solvent remained above the adsorbent. Methanol was employed as the solvent in all stages. The crude streptothricin hydrochloride was dissolved in methanol at a concentration of 10–50% and the solution was allowed to flow through the adsorbent layer under a pressure of 10–20 mm. of mercury. When the solution was all in the adsorbent layer, the column was developed with methanol under pressure. The eluates were tested for presence of solids, the eluates were collected, and the products were obtained as described in the section on chromatography with aluminum oxide. Data of four experiments are in Table V.

Streptothricin Helianthate.—Twenty grams of streptothricin hydrochloride, 455 units/mg. activity, was dissolved in 200 cc. of methanol and a hot solution of 16 g. of methyl orange in 160 cc. of water was added with continuous stirring. After stirring for one hour, the mixture was kept four hours at 5°. The orange-colored precipitate of streptothricin helianthate was removed by centrifuging and dissolved in 200 cc. of methyl alcohol at 65°. The solution was filtered while hot, and 200 cc. of hot water was added. Scratching induced crystallization. After twelve hours at 5°, the crystals were removed by filtration, washed once with water, and dried *in vacuo* to yield 17.15 g. of streptothricin helianthate, 340 units/mg. activity.

Two such samples were recrystallized twice from aqueous methanol and dried for two hours at 100° *in vacuo* for analysis. When heated on the micro-block the samples melted with decomposition between 225–230°.

Anal. Found: (Sample I) C, 50.36; H, 5.14; N, 16.86. (Sample II) C, 50.40; H, 5.44; N, 16.84.

Conversion of Streptothricin Helianthate to Streptothricin Hydrochloride.—Seven grams of the helianthate was added to 30 cc. of methyl alcohol, followed by 1.2 cc. of concentrated hydrochloric acid dropwise with constant stirring. After triturating thoroughly, the violet colored mixture, which had a *p*H of 4.0, was filtered through a 1-g. pad of Darco G-60 and the Darco was washed with three 3-cc. portions of methyl alcohol. The colorless filtrate was treated immediately with 175 cc. of acetone and the resulting precipitate removed by centrifuging. The precipitate was washed once with acetone, and dried at 25° *in vacuo* to yield 2.55 g. of a white powder, 830 units/mg. activity, $[\alpha]^{25}D$ −51.3° (*c*, 1.4 in water).

Purification of Streptothricin by Precipitation with Sodium Phosphotungstate.—The sodium phosphotungstate solution used in the experiment described below was prepared by adjusting a 10% aqueous solution of phosphotungstic acid to *p*H 6.8 with 2.5 *N* sodium hydroxide.

A 4.0-g. sample of streptothricin formate (60 units/mg. activity) was dissolved in 100 cc. of distilled water and 36 cc. of sodium phosphotungstate solution was added during continuous stirring. The resulting precipitate was removed by centrifuging, and was washed with 50 cc. of water. After centrifuging, the precipitate was added to 250 cc. of 2 *N* phosphoric acid in acetone. After centrifuging, the precipitate was washed with 250 cc. of water and the mixture was centrifuged. The precipitate was added to 800 cc. of 4% 2 *N* sulfuric acid in acetone. After stirring thirty minutes, the mixture was centrifuged, and the precipitate was washed with 800 cc. of water. The mixture was centrifuged to yield a precipitate (A) and the supernatant solution. The *p*H of this solution was kept at 3.0 while concentrating *in vacuo* to 30-cc. volume. Five volumes of methyl alcohol was added, and the precipitate was removed by centrifuging. After drying *in vacuo* the streptothricin sulfate concentrate (B) weighed 240 mg. and showed 350 units/mg. activity.

The precipitate (A) was added to 400 cc. of 4% 2 *N* sulfuric acid in acetone. After stirring the mixture thirty minutes, it was centrifuged, and the precipitate was washed with 400 cc. of water. After centrifuging, the supernatant solution yielded 40 mg. of streptothricin sulfate when treated as described for the prior supernatant solution. This concentrate (C) showed 400 units/mg. activity and the total yield of concentrate was 40% in terms of activity.

The results of other experiments using this procedure are presented in Table VI.

TABLE V

CHROMATOGRAPHIC PURIFICATION OF STREPTOTHRICIN WITH DARCO G-60

Concentrate used		Adsorbent		Eluates		Product		
Wt., g.	Activity units/mg.	Darco G-60, g.	Filter paper pulp, g.	Number	Volume, ml.	Wt., g.	Activity units/mg.	% Activity recovered
4.0	49	12	6	1-2	55[a]	0.836	5	2
				3	20	.472	59	14
				4	20	.420	131	28
				5	50	.605	118	37
				6-9	304[a]	.544	21-93[b]	17
								—
								98
2.76	120	42	35	1-3	70[a]	.959	*ca.* 40	13
				4-5	42[a]	.570	119-143[b]	23
				6-7	43[a]	.381	181-191[b]	21
				8-14	152[a]	.431	212-253[b]	28
								—
								85
8.0	300	250	180	1-2	208[a]	1.06	250-421[b]	13
				3	405	3.00	480	60
				4-6	895[a]	1.80	110-385[b]	23
								—
								96
2.5	430	10	5	1	20	0.62	730	42
				2	100	1.39	460	60
								—
								102

[a] Combined volumes. [b] Average activities of highest and lowest activity fractions.

TABLE VI

STREPTOTHRICIN SULFATE CONCENTRATE FROM PHOSPHOTUNGSTATES

Concentrate used		Yield of streptothricin sulfate				
		Fraction (B)		Fraction (C)		
Wt., g.	Activity, units/mg.	Wt., g.	Activity, units/mg.	Wt., g.	Activity units/mg.	% Activity recovered
43.7	22	2.34	200	1.10	220	64
7.5	185	3.32	250	0.42	330	70
9.0	185	4.20	260	0.65	300	55
42.0[a]	130	11.96	170	8.34	250	75

[a] Another fraction was obtained in this experiment by repeating the last two steps. It weighed 1.20 g. and showed 260 units/mg. activity (total recovery of activity 81%).

A modification of the procedure described above gave similar results. In this case, 4% 2 *N* hydrochloric acid in acetone was substituted for the sulfuric acid–acetone mixture, and the streptothricin hydrochloride was precipitated with acetone.

Purification of Streptothricin by Precipitation with Picrolonic Acid.—A 6.0-g. quantity of streptothricin hydrochloride, 450 units/mg. activity, $[\alpha]^{25}D$ −51° (*c*, 0.8 in water) was dissolved in 200 cc. of water. After cooling to 2°, the *p*H was adjusted to 10.8 by adding 10 cc. of cold 2.5 *N* sodium hydroxide dropwise. A hot 10% solution of pure picrolonic acid in 95% ethyl alcohol was added immediately until there was no further precipitation. The *p*H at this point was *ca.* 5.0. After standing for several hours at 5°, the precipitate of streptothricin picrolonate was collected by filtration and dissolved in a warm mixture of 100 cc. of 50% ethyl alcohol and 25 cc. of 95% ethyl alcohol. The solution was filtered to remove a small amount of insoluble material and allowed to cool slowly. During cooling, a small amount of flocculent precipitate formed which was removed by filtration. Crystals in the form of rosets appeared after one-half hour. After several hours at 5°, the crystalline salt was removed by filtration. A small sample, after drying *in vacuo* showed 300 units/mg. activity.

The streptothricin picrolonate was recrystallized by dissolving in hot acetone and adding hot water to incipient turbidity. It was recrystallized again from 50% ethyl alcohol.

The product was converted to streptothricin hydrochloride by dissolving it in a minimum of acetone and adding 2.5 cc. of concd. hydrochloric acid with stirring. Acetone was added, the mixture was centrifuged, the supernatant liquid was decanted, and the precipitate was dissolved in 10 cc. of methyl alcohol and reprecipitated with acetone. After washing with acetone to remove traces of picrolonic acid and drying at room temperature *in vacuo*, 1.75 g. of white powder was obtained; 670 units/mg. activity, $[\alpha]^{25}D$ −54° (*c*, 2.4 in water).

The utility of picrolonic acid for the purification of streptothricin was not limited to preparations of high activity. The results obtained in several experiments employing concentrates of varying activities are summarized in Table VII. The picrolonate was recrystallized or precipitated once from 50% ethyl alcohol in these experiments. Although crystalline picrolonates were not always obtained from concentrates of less than 350 units/mg. activity, a definite increase in purity was always found.

TABLE VII

Streptothricin formate concentrate used		Yield of hydrochloride after conversion from the picrolonate		
Wt., g.	Activity, units/mg.	Wt., g.	Activity, units/mg.	% Activity recovered
12.0[a]	300	4.50	580	73
6.0	300	2.80	450	65
8.0	300	3.85	510	82
5.8	315	2.40	610	85
6.0[a]	200	1.90	510	85
3.0[a]	425	1.60	650	87
2.0	200	0.48	460	58

[a] These preparations yielded crystalline streptothricin picrolonates.

Purification of Streptothricin by Precipitation of a Crystalline Reineckate.—A 400-mg. quantity of streptothricin hydrobromide, 600 units/mg. activity, was dissolved in 10 cc. of water and a saturated solution of Reinecke acid added until no further precipitate was obtained. The precipitate was partly crystalline. It was removed by centrifuging and washed with three 2-cc. portions of cold water. After extracting with 15 cc. of water at 45° for one-half hour, the insoluble portion was removed by centrifuging. As the supernatant liquid cooled slowly, fine needles of streptothricin reineckate deposited; 300 units/mg. activity. A second extraction of the insoluble reineckate with 12 cc. of water at 45°, followed by centrifuging and cooling the supernatant liquid yielded another crop of the crystalline reineckate.

The crystalline streptothricin reineckate was transformed into the hydrobromide by dissolving it in 15 cc. of warm water and adding an aqueous 2.5 *N* pyridine hydrobromide solution until no further precipitate of pyridine reineckate occurred. The pyridine reineckate was removed by filtration and the filtrate was concentrated to dryness at reduced pressure. The residue was dissolved in 2 cc. of methyl alcohol, and an excess of acetone added. The resulting precipitate was removed by centrifuging and dried to yield 150 mg. of streptothricin hydrobromide, 650 units/mg. activity.

The original solution and mother liquors from which the streptothricin reineckate precipitated were combined and treated with an aqueous 2.5 *N* pyridine hydrobromide solution to yield an additional 110 mg. of hydrobromide, assaying 470 units/mg. activity.

Pyridine hydrochloride has been used also to convert the reineckate to streptothricin hydrochloride.

Summary

Streptothricin hydrochloride which has shown 830 units/mg. activity and $[\alpha]^{25}D$ −51.3° has been isolated from culture broths of *Streptomyces lavendulae*. The following sequence of steps was used to produce this product from the broth: charcoal adsorption, elution with formic acid, precipitation with picric acid and direct conversion to hydrochlorides, chromatography of hydrochlorides, helianthate, hydrochloride.

The following additional precipitants have been used to effect purification of streptothricin concentrates: picrolonic acid, flavianic acid, Reinecke salt and phosphotungstic acid.

RAHWAY, NEW JERSEY RECEIVED JANUARY 22, 1946

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, MERCK & CO., INC.]

Streptomyces Antibiotics. VII. The Structure of Streptidine

BY ROBERT L. PECK, CHARLES E. HOFFHINE, JR., ELIZABETH W. PEEL, ROBERT P. GRABER, FREDERICK W. HOLLY, RALPH MOZINGO AND KARL FOLKERS

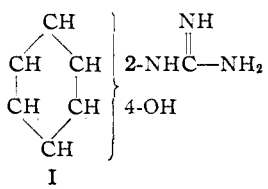
Streptidine is one of the meso forms of 1,3-diguanido-2,4,5,6-tetrahydroxycyclohexane according to present experimental evidence.

Streptidine[1,2] was characterized as an optically inactive, hydroxylated, strongly basic substance having the molecular formula $C_8H_{18}N_6O_4$. No carboxyl, carbonyl or typical primary amino groups were present. Further tests showed the absence of O-, N- and C-methyl groups. Streptidine was found to have eight acetylatable –OH and >NH groups and two basic groups.

The two basic groups of streptidine were shown by potentiometric titration to be of an order of basic strength equivalent to guanidine. The presence of guanido groups in streptidine was proved by degradation of streptidine to guanidine. Streptidine was oxidized in aqueous solution with potassium permanganate, and the liberated guanidine was isolated as the picrate. The yield of the picrate corresponded to 1.3 moles of guanidine per mole of streptidine. Thus, there are two guanido groups in streptidine. These two guanido groups account for all six nitrogen atoms and the strongly basic character of streptidine.

Four of the eight acetyl groups in octaacetylstreptidine[1] can now be considered as N-acetyl groups on the basis of the formation of diacetyl derivatives of monosubstituted guanidines under the experimental conditions used. The remaining four acetyl groups of octaacetylstreptidine would then appear to be O-acetyl groups. Thus, all four oxygen atoms of streptidine are apparently present as hydroxyl groups.

The analytical data[1] on several salts showed that streptidine has two hydrogen atoms less than that number required for a saturated acyclic compound. The infrared and ultraviolet absorption spectra[1] of streptidine did not show bands suggestive of a C=C group. Furthermore, there was no evidence of unsaturation when bromine water was added to an aqueous solution of streptidine dihydrochloride. Thus, streptidine appears to have the carbocyclic structure (I) in which the two guanido groups are in the 1,2-, 1, 3- or 1,4-positions.



I

Each of the remaining four carbon atoms of the ring appears to have one hydroxyl group. Further information on the structure was obtained by a study of the reactions of the products formed by alkaline hydrolysis of streptidine.

Alkaline hydrolysis of streptidine involved two

(1) Peck, Graber, Walti, Peel, Hoffhine and Folkers, THIS JOURNAL, **68**, 29 (1946).

(2) Brink, Kuehl and Folkers, *Science*, **102**, 506 (1945).