points mentioned above will appear in a paper[13] to be submitted to *Inorganic Chemistry*.

(13) J. V. Silverton and J. L. Hoard; the unequivocal assignment of the antiprismatic configuration to zirconium(IV) acetylacetonate will be included.

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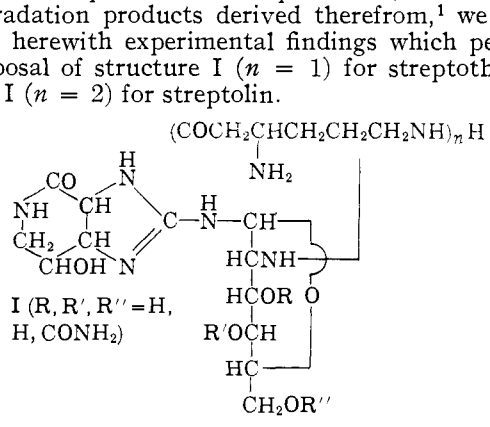
RECEIVED SEPTEMBER 16, 1961

CONSTITUTION OF THE STREPTOLIN-STREPTOTHRICIN GROUP OF *STREPTOMYCES* ANTIBIOTICS

Sir:

As a climax to our studies on the nature of the water-soluble, non-crystalline *streptomyces* antibiotics streptolin and streptothricin, as well as degradation products derived therefrom,[1] we present herewith experimental findings which permit proposal of structure I (n = 1) for streptothricin and I (n = 2) for streptolin.



Quantitative determination of the drastic acid hydrolysis products of streptolin provided these results (moles of product/moles of antibiotic)

Carbon dioxide 1.02; ammonia	0.88
Gulosamine (A) + anhydrogulosamine (B)	0.19
L-β-Lysine (C)	1.24
Streptolidine (D)	0.46
N-(L-β-Lysyl)L-β-lysine (E)[2]	0.34
N-*guan*-Streptolidylgulosaminide (F)	0.44

Hydrolysis of streptothricin produces *ca.* 0.9 mole of product (C) and *no* (E).[3] Addition of the molecular formulas of these degradation products leads to the assignments $C_{25}H_{46}N_{10}O_9$ for streptolin and $C_{19}H_{34}N_8O_8$ for streptothricin, compatible with elemental determinations on various salts.[4] Although not a carboxylic acid, streptolin possesses four basic centers, one guanidino and three aliphatic amino groups (pK_a's 7.5, 8.4, 9.3 and 10.6 in water); streptothricin possesses one less amino group (pK_a's 7.1, 8.2 and 10.1 in water). Van Slyke determination showed all of the nonguanidino amino groups to be primary. Periodate uptake of the antibiotics was negligible (*e.g.*, streptolin consumed 0.2 mole in 10 minutes, and 0.7 mole in 24 hours); O-, N- and C-methyl groups are absent. By thiosemicarbazide determination, it was shown that neither antibiotic behaved as an aldehyde or ketone.

On standing in 1 N hydrochloric acid at room temperature for several days, streptolin was converted to a biologically-inactivated material, streptolinic acid (II), purified by cellulose column chromatography (found for $C_{25}H_{48}N_{10}O_{10}\cdot 5/2H_2SO_4$: C, 33.61; H, 6.27; N, 15.22; S, 9.8. On complete hydrolysis, this new substance gave rise to all the characteristic degradation products (including carbon dioxide and ammonia) secured from streptolin itself. Streptolinic acid[5] possesses one carboxyl group, pK_a 2.0 (water), (but is not an α-amino acid, as shown by quantitative ninhydrin determination) and five basic centers, four of which were shown by Van Slyke assay (6.1%) to be primary amino. Streptolinic acid readily consumed approximately one mole of periodate, generating formaldehyde and ammonia. On reductive methylation (CH_2O; H_2; Pd-on-C in aqueous methanol), the parent antibiotic yielded, after drastic acid hydrolysis, compounds (D) and (F), but not (C) and (E). On the other hand, II on similar treatment did not give rise to any of these characteristic degradation products. These findings, together with other pertinent information described below, indicate that (i) the two non-guanidino amino groups in the streptolidyl gulosaminide portion of the antibiotic are masked, and (ii) a lactam ring in the streptolidine unit of the intact antibiotic must be present, which in the conversion of streptolin to II is opened hydrolytically.

That the amino groups in the β-lysine portion are free in the antibiotic was confirmed in the following way.[6] Reductive methylation of either streptolin or dipeptide (E), then drastic acid hydrolysis, produced the same pair of N-methylated β-lysines [regarded as β-N,N-dimethyl and β-N,N-ϵ-N,N-tetramethyl], as shown by paper chromatographic studies.

In consideration of the foregoing, attachment of the β-lysyl or β-lysyl-β-lysyl unit to the streptolidyl gulosaminide moiety must be by way of the amino group in the hexose portion.[6]

The obligatory incorporation of the elements of carbon dioxide and ammonia into an O-carboxamido unit, attached to the gulosamine portion, is supported by these diagnostic determinations. Under suitable hydrolytic conditions, carbon dioxide and ammonia are liberated from both streptolin and streptothricin in a characteristic fashion, very similar to that observed in the case of novobiocin, an authentic urethan. On treatment with nitrous acid, both streptolin and novobiocin give rise to substantial amounts of carbon dioxide (under similar conditions, compounds (F) and (C) generate only negligible amounts of this gas). Although assignment of the carboxamido unit to one of the ring oxygens might appear to be in order by reason of the behavior of the antibiotic with periodate,

(1) See accompanying Communications, *J. Am. Chem. Soc.*, (1961), and preceding papers in this series.

(2) Assignment of structure to the peptide is based on (details to be published): (i) correct elemental analysis of the tri *p*-hydroxyazobenzene-*p'*-sulfonate, (ii) molecular weight by titration, (iii) hydrolysis to β-lysine (only), and (iv) comparison of pK measurements with those of model compounds, indicating ϵ-attachment of the second lysine unit to the first (electrometric titrations and interpretations by Dr. H. Boaz, Eli Lilly and Co.).

(3) Geomycin yields on total hydrolysis four moles of β-lysine: H. Brockmann and H. Musso, *Ber.*, **88**, 648 (1955).

(4) To be published.

(5) A similar substance, with similar properties, can be secured by acid inactivation of streptothricin.

(6) A similar conclusion regarding roseothricin A was reached, through other means, by T. Goto, Y. Hirata, S. Hosoya and N. Komatsu, *Bull. Chem. Soc. Japan*, **30**, no. 7, 729 (1957).

our observation that N,N-diacetyl (F) also takes up this oxidant only slowly[7] indicates that this conclusion is not justified.

In view of the close similarity of streptolin–streptothricin and certain *streptomyces* antibiotics such as roseothricin, geomycin and others, we suggest that the general expression I[8] may be applicable to these other members of this ubiquitous group.

Acknowledgment.—Structural investigations on streptolin, streptothricin and degradation products were supported by Research Grant G-4118 and E-618 (University of Illinois) and E-585 (University of Wisconsin) from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service; and Contract (Nonr1202(10) (University of Wisconsin) with the Office of Naval Research.

(7) Considered to be a conformational effect, caused by the presence of diaxial hydroxyl groups.

(8) The structure I established herein differs in several important respects from the proposal for roseothricin-A by Goto, *et al.* (footnote 6), and the more recent structural suggestion for the closely related racemomycin-O (S. Takemura, *Chem. and Pharm. Bull.*, **8**, 578 (1960).

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STREPTOTHRICIN AND STREPTOLIN: THE STRUCTURE OF STREPTOLIDINE (ROSEONINE)

Sir:

On acid hydrolysis, the *streptomyces* antibiotics streptothricin[1] and streptolin[2] yield carbon dioxide, ammonia, L-β-lysine,[3,4] 2-amino-2-deoxy-α-D-gulose,[5] and a basic amino acid, streptolidine[6] (I) the structure of which now has been elucidated.[10]

(1) H. E. Carter, R. K. Clark, Jr., P. Kohn, J. W. Rothrock, W. R. Taylor, C. A. West, G. B. Whitfield, and W. G. Jackson, *J. Am. Chem. Soc.*, **76**, 566 (1954).

(2) E. E. Smissman, R. W. Sharpe, B. F. Aycock, E. E. van Tamelen, and W. H. Peterson, *ibid.*, **75**, 2029 (1953).

(3) H. E. Carter, W. R. Hearn, E. M. Lansford, Jr., A. C. Page, Jr., N. P. Salzman, D. Shapiro, and W. R. Taylor, *ibid.*, **74**, 3704 (1952).

(4) E. E. van Tamelen and E. E. Smissman, *ibid.*, **74**, 3713 (1952).

(5) E. E. van Tamelen, J. R. Dyer, H. E. Carter, J. V. Pierce, and E. E. Daniels, *ibid.*, **78**, 4817 (1956).

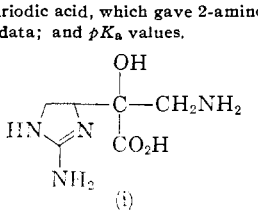
(6) Streptolidine is the trivial name assigned to a basic amino acid present in the acid hydrolysate of streptolin.[2,7] Streptolidine has been shown to be identical with a basic amino acid present in the acid hydrolysate of streptothricin,[1,8] originally termed compound B.[9]

(7) Glenn Brewer, Ph.D. Thesis, University of Wisconsin, 1953.

(8) Charles C. Sweeley, Ph.D. Thesis, University of Illinois, 1955.

(9) Charles A. West, Ph.D. Thesis, University of Illinois, 1952.

(10) By direct comparison, streptolidine has been shown to be identical with roseonine, derived from roseothricin,[11-13] geamine, derived from geomycin,[14-16] and a basic amino acid derived from racemomycin[17] and mycothricin.[18] A structural formula (i) has been proposed[11,12] for roseonine based primarily on oxidation, which gave glycine and guanidine; degradation using silver nitrite followed by phosphorus and hydriodic acid, which gave 2-amino-4-ethyl-imidazole; periodate oxidation data; and pK_a values.



(i)

Cellulose and charcoal chromatography of streptothricin and streptolin hydrolysates yielded I as a crystalline hydrochloride, m.p. 173–190° dec., $[\alpha]^{22}D$ +56.8° (*c* 2.35, water). (Found: C, 27.97; H, 5.20; N, 21.55; Cl, 27.03). Streptolidine showed pK_a values of 2.5, 8.72, and 11.3 (water) and 3.95, 9.10, and 12.65 (66% dimethylformamide)[19]; it gave positive Weber and ninhydrin tests and negative Sakaguchi, Ehrlich, Benedict, Schiff, Elson–Morgan, and biuret tests. Van Slyke analysis indicated one primary amino function; C-methyl, O-methyl, N-methyl, and α-amino acid groups were shown to be absent. Streptolidine consumed one mole of periodate rapidly, giving in addition to formaldehyde and ammonia, an unstable *aldehyde* (II) $[\alpha]D$ +108° (*c* 1.0, water) (positive Benedict, Tollens, and Schiff tests).[20] Treatment of I with excess periodate for an extended period of time gave glycocyamidine. The N-2,4-dinitrophenyl derivative of I, m.p. 202–208° (Found: C, 40.53; H, 3.94; N, 23.43) did not consume lead tetraacetate[21]; thus I is not an α-hydroxyacid.[20] That the aliphatic amino group is terminal was suggested most directly by the earlier observation that glycine was produced on oxidation of streptolidine,[11] and this view is now supported by the formation of terminal methyl product (Found: C—CH_3, 2.67) on reductive deamination[22] of chromatographically pure but non-crystalline mono-(N-benzenesulfonyl)-streptolidine (Found: C, 43.21; H, 4.72; C–CH_3, 0.0). Thus streptolidine is a 2-aminoimidazoline having the substituent groups —CO_2H and —CHOH—CH_2NH_2.

Sodium borohydride reduction of the aldehyde II furnished the crystalline hydroxyacid III, m.p. 250° dec., $[\alpha]D$ +93.2° (*c* 3.10, water) (Found: C, 37.70; H, 5.86; N, 26.21). Treatment of III with methanolic hydrogen chloride gave an ester[23] (λ_{max} 5.75 μ), hydrogenation of which produced the *diol* IV, $[\alpha]D$ +40° (*c* 1.18, water), flavianate, m.p. 234–235° (Found: C, 39.24; H, 3.64; N, 15.69), which did not reduce periodate.[20] Hence III is *trans*-2-amino-5-hydroxymethylimidazoline-4-carboxylic acid. DL-*trans*-2-Amino-5-hydroxymethylimidazoline-4-carboxylic acid (V) was secured as follows. Alkaline hydrolysis and epimerization of 3,4-(1′,3′-dibenzyl-2′-ketoimidaz-

(11) K. Nakanishi, T. Ito, M. Ohashi, I. Morimoto, and Y. Hirata, *Bull. Chem. Soc. Japan*, **27**, 539 (1954).

(12) K. Nakanishi and M. Ohashi, *ibid.*, **30**, 725 (1957).

(13) T. Goto, Y. Hirata, S. Hosoya, and N. Komatsu, *ibid.*, **30**, 729 (1957).

(14) H. Brockmann and H. Musso, *Naturwissenschaften*, **41**, 451 (1954).

(15) H. Brockmann and H. Musso, *Angew. Chem.*, **67**, 167 (1955).

(16) H. Brockmann and H. Musso, *Ber.*, **88**, 648 (1955).

(17) H. Taniyama and S. Takemura, *Yakugaku Zasshi*, **77**, 1215 (1957) (*C.A.*, **52**, 3886*i* (1958)).

(18) G. Rangaswami, C. P. Schaffner, and S. A. Waksman, *Antibiotics and Chemotherapy*, **6**, 675 (1956).

(19) We are grateful to Dr. Harold Boaz, Eli Lilly and Co., for determining the pK_a values.

(20) This experimental finding is inconsistent with structure (i) (ref. 10).

(21) Observed by Dr. C. R. Narayanan, University of Wisconsin.

(22) A. Nickon and A. Sinz, *J. Am. Chem. Soc.*, **82**, 753 (1960).

(23) Similar treatment of *cis*-2-amino-5-hydroxymethylimidazoline-4-carboxylic acid, m.p. 144–146°, derived from reaction of *erythro*-2,3-diamino-4-hydroxybutyric acid with cyanogen bromide, furnished a lactone (λ_{max} 5.67 μ).