to the dilute solution. This would then be a direct measure of the thermodynamic stability of the ordered form.

We have investigated the optical rotation (at 546 mμ) as a function of temperature for ribonuclease (Sigma, Chromatographed Grade Type II) dissolved in aqueous solutions of increasing lithium bromide concentration. The results are summarized in Fig. 1. In pure water the variation of the specfic rotation with temperature is virtually identical with that previously reported.[1,10,11,12] The structural transformation, characterized by a relatively sharp increase in levorotation, is clearly discernible and terminates at approximately 70°. The addition of lithium bromide results in a progressive lowering of the transition temperature, indicating a decreased stability of the ordered structure present in the native form. The increase in levorotation during the transformation is still noted but the magnitude of the change in the specific rotation decreases with increasing lithium bromide concentration. For a 4.5 *M* lithium bromide solution the transformation temperature has been lowered to below 15°. It can be concluded that in the salt concentration range studied, lithium bromide acts as a universal transformer of the ordered structures of polypeptides and proteins. The apparent discrepancy between the results for the fibrous proteins and those for proteins in dilute solution consequently is eliminated. A continuity in the phase diagram, as the protein concentration is varied, can now be anticipated.

At any fixed temperature, as the concentration of lithium bromide increases, the optical rotation becomes less levorotatory, in accord with the observations of Harrington and Schellman,[1] this effect being more marked in the transformed state. The transformed state has been identified with a random-coil chain conformation in pure water.[10,12] The continuity of the data presented indicates that this must also characterize the transformed state obtained after the addition of lithium bromide. Thus, in this case a substantial decrease in levorotation with increasing lithium bromide concentration does not necessarily reflect the stabilization or formation of helical structures.

As has been pointed out previously,[13,14] specific solvent or medium effects can in principle change the observed optical rotation without the necessity of any concomitant structural changes occurring within the molecule. When this situation exists, an erroneous conclusion can be made when the optical rotation measurements are limited to a single temperature. This appears to be the case for the interaction of lithium bromide with proteins and polypeptides. Though the levorotation decreases, a decrease in the thermodynamic stability of the ordered structures also manifests itself. Any interpretation of changes in optical rotation based solely in terms of structural changes is, therefore, in a tenuous position in general, and erroneous in the specific case under consideration.

In addition, a survey of data in the literature shows that the addition of lithium bromide to solutions of polypeptides and proteins always

(13) W. Kauzmann, *Ann. Rev. of Physical Chem.*, **8**, 413 (1957).
(14) W. Kauzmann, *Adv. in Prot. Chem.*, **14**, 1 (1959).

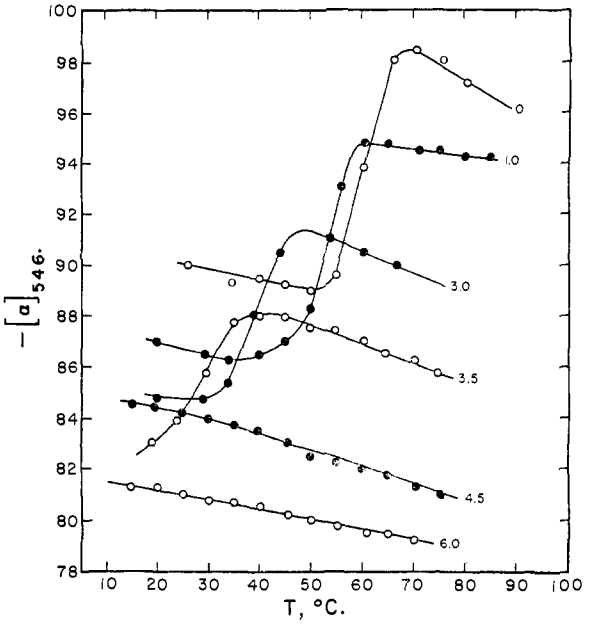

Fig. 1.—Plot of specific rotation at 546 mμ ($[\alpha]_{546}$) as a function of temperature for a 1.4% ribonuclease solution dissolved in aqueous lithium bromide solution of indicated molarity.

results in a decreased levorotation.[1,2,15-19] It is particularly pronounced in the case of gelatin wherein the random coil form is maintained.[15,16] From this point of view, also, the difficulties experienced in interpreting the changes in optical rotation and accompanying inactivation of pepsin with lithium bromide can be clarified.[19]

More details of the present work, together with the complexities observed at higher lithium bromide concentration,[4,5] will be presented subsequently. Interpretation will be made in the context of the universal action of lithium bromide and similar compounds in coöperatively disrupting the ordered structures of the fibrous and globular proteins over the complete range of polymer concentration.

(15) D. C. Carpenter and F. E. Lovelace, *J. Am. Chem. Soc.*, **57**, 2337 (1935).
(16) W. F. Harrington, *Nature*, **181**, 997 (1958).
(17) W. F. Harrington and M. Sela, *Biochim. et Biophys. Acta*, **31**, 427 (1959).
(18) I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela, and E. Katchalski, *J. Am. Chem. Soc.*, **82**, 5263 (1960).
(19) G. E. Perlmann, "Proceedings of the Fourth International Congress of Biochemistry," Vol. IX, Pergamon Press, New York, N. Y., 1959, p. 32.

POLYMER STRUCTURE SECTION L. MANDELKERN
NATIONAL BUREAU OF STANDARDS
WASHINGTON 25, D. C. D. E. ROBERTS

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THE CONFIGURATION OF $Zr(C_2O_4)_4^{-4}$ AND THE STEREOCHEMISTRY OF DISCRETE EIGHT-COORDINATION[1]

Sir:

The configurations affording a superior stereochemistry for discrete eight-coördination com-

(1) Supported in part by the Army Research Office (Durham), the National Science Foundation, and the Advanced Research Projects

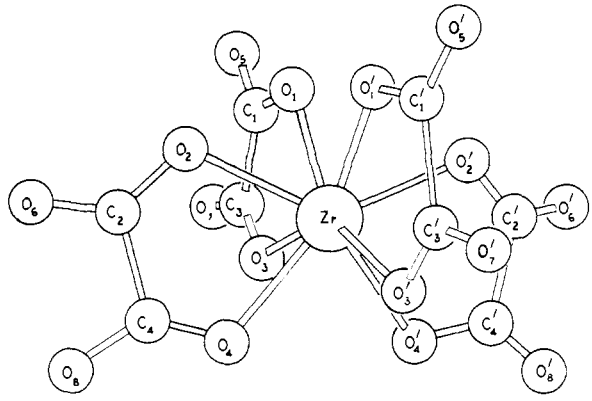

Fig. 1.—Idealized ($\bar{4}$m2) model of $Zr(C_2O_4)_4^{-4}$: the mutually perpendicular mirror planes, in which all atoms lie, intersect in the (vertical) $\bar{4}$-axis. The inner coördination group is most easily visualized in terms of the structurally equivalent trapezoids delineated by $O_3O_1O_1'O_3'$ and $O_2O_4O_4'O_2'$.

plexes are, as pointed out in the first definitive structural paper[2] dealing with this subject, the dodecahedron with triangular faces characterizing the bond arrangement in $Mo(CN)_8^{-4}$ and $W(CN)_8^{-4}$, the square or Archimedean antiprism established[3] shortly thereafter for TaF_8^{-3}, and, as an interesting but unobserved possibility, the configuration[2] which lies halfway between those[4,5] of $NbF_7^{=}$ and $Nd(OH_2)_9^{+3}$. We wish now to report that the tetrakis-oxalato complexes of Zr(IV) and Hf(IV) in the sodium salts are excellent examples of the $Mo(CN)_8^{-4}$ configurational type. We wish equally to challenge the widely held impression[6] that the square antiprism enjoys a dominating position in the stereochemistry of eight-coördination. Rather it appears that approximately equal *a priori* probabilities are to be assigned to the $Mo(CN)_8^{-4}$ and antiprismatic types.

Objective analysis by methods previously outlined[7,8] of three-dimensional spectrometrically recorded X-ray data from a single crystal of $Na_4[Zr(C_2O_4)_4]\cdot 3H_2O$ provides the configuration for $Zr(C_2O_4)_4^{-4}$ (and the virtually isodimensional $Hf(C_2O_4)_4^{-4}$) shown in Fig. 1. The four-molecule orthorhombic unit has $a = 7.45$, $b = 11.83$, $c = 19.75$ Å. Calculated and measured densities are, respectively, 2.30 and 2.27 g./cc. The space group is $B22_12$. Intensity counts were taken with Mo $K\alpha$ radiation for all forms $\{hkl\}$ having $(\sin\theta)/\lambda < 0.96$. Results now presented are based upon the 1774 forms (1653 above background) having $(\sin\theta)/\lambda < 0.75$. After partial refinement by difference syntheses, the discrepancy index presently stands at 0.096.

The twofold axis required of $Zr(C_2O_4)_4^{-4}$ by the space group coincides with the $\bar{4}$-axis of the idealized $Mo(CN)_8^{-4}$ configuration. All carboxyl groups and half the oxalato rings are flat within experimental accuracy; the remaining rings depart trivially from planarity. The most obvious deviation from $\bar{4}$m2 symmetry is in the angle between the mean planes of the ring systems: 1.5° from an exact right angle. Averaged bond distances are Zr–O, 2.215; C–C, 1.56; C–O, 1.26 and 1.22 Å. for bonds lying, respectively, within and external to the rings. Averaged bond angles within the rings are 71.8° at zirconium, 119.6° at oxygen, and 114.2° at carbon. The O–Zr–O angle between adjacent rings in the same plane is 69.7°.

No decisive choice between the $Mo(CN)_8^{-4}$ and antiprismatic configurations emerges from the consideration of steric repulsions between ligands. Taking the energy as proportional to R^{-n}, calculation indicates stability for the antiprism when $n > 7$. We then note that empirical data suggest seven as the value of the Born exponent appropriate to the usual (neon shell) ligands.[9] The assumption of the $Mo(CN)_8^{-4}$ configuration (with $\bar{4}m2$ symmetry required in the crystal) by a bis-diarsinetitanium(IV) tetrachloride[10] with argon-shell ligands ($n = 9$) is thus in obvious (but not really serious) violation of prediction.

Application of ligand field theory in the strong field approximation appropriate to the strongly covalent complexes of interest is similarly indecisive. The non-bonding pair of electrons in the diamagnetic complex of an atom such as Mo(IV) or U(IV) has at its disposal, for each configuration, a d-orbital uniquely suited to minimize interaction with the ligands. This conclusion, indeed, already is evident in the valence bond treatment[11] of hybridized orbitals for the central atom. The "bond strengths" given by the calculations[11] imply rather similar bond lengths and energies for the $Mo(CN)_8^{-4}$ and antiprismatic configurations. Elimination of the extra electron pair so as to deal with Zr(IV), Ta(V) and their congeners should not, and apparently does not, favor one or the other configuration disproportionately; but it does lend some measure of theoretical interest[12] to the unobserved third configuration[2] mentioned earlier.

Experimental fact provides the essential comparison between the two observed configurations: the count of certainly established examples of the $Mo(CN)_8^{-4}$ type presently is somewhat higher than for the antiprism. Documentation of this statement and further elucidation of the several

Agency. Machine computations were carried out at the Cornell Computing Center, Mr. Richard C. Lesser, Director.

(2) J. L. Hoard and H. H. Nordsieck, *J. Am. Chem. Soc.*, **61**, 2853 (1939).

(3) J. L. Hoard, W. J. Martin, M. E. Smith and J. F. Whitney, *ibid.*, **76**, 3820 (1954). Presented at Sixth Annual Symposium of the Division of Physical and Inorganic Chemistry of the American Chemical Society, Columbus, Ohio, December, 1941.

(4) J. L. Hoard, *ibid.*, **61**, 1252 (1939).

(5) L. Helmholz, *ibid.*, **61**, 1544 (1939).

(6) *Cf.* R. J. Gillespie in "Advances in the Chemistry of the Coordination Compounds," Stanley Kirschner, The Macmillan Company, New York, N. Y., 1961, pp. 34–39.

(7) J. L. Hoard, M. Lind and J. V. Silverton, *J. Am. Chem. Soc.*, **83**, 2770 (1961).

(8) J. L. Hoard, B. Pedersen, S. Richards and J. V. Silverton, *ibid.*, **83**, 3533 (1961).

(9) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 3rd ed., 1960, p. 509.

(10) J. Dollimore, P. Pauling and G. Robertson, Abstract G-1, Amer. Crystal. Assn. Meeting in Boulder, Colo., July 31–Aug. 4, 1961.

(11) G. Racah, *J. Chem. Phys.*, **11**, 214 (1943); G. H. Duffey, *ibid.*, **18**, 746 and 1444 (1950).

(12) *Cf.* G. E. Kimball, *ibid.*, **8**, 188 (1940).

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.

DEPARTMENT OF CHEMISTRY J. L. HOARD
CORNELL UNIVERSITY G. L. GLEN
ITHACA, NEW YORK J. V. SILVERTON

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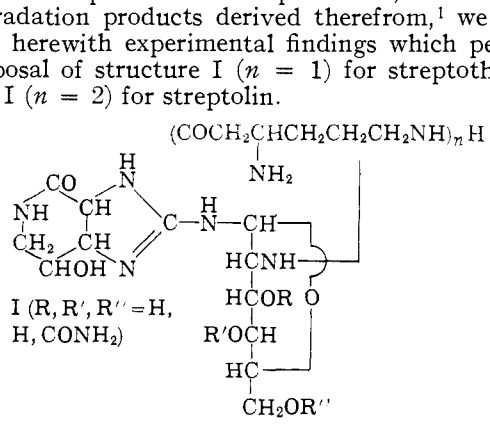
CONSTITUTION OF THE STREPTOLIN-STREPTOTHRICIN GROUP OF *STREPTOMYCES* ANTIBIOTICS

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 p*K* 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).