1α,5α-Epidithio-17a-oxa-D-homoandrostane-3,17-dione.—6D-Homo-17a-oxa-1,4-androstandiene-3,17-dione, 2.00 g., was dissolved in 60 ml. of pyridine and hydrogen sulfide was bubbled into the solution for two hours. Then 2 drops of piperidine was added and the reaction was allowed to stand for 3 days. After this time 200 ml. of water was added and the solution was extracted three times with ether. A solid formed in the aqueous layer and was separated by filtration. It was crystallized from ethyl acetate to give 0.34 g. of 1α,5α-epidithio-17a-oxa-D-homoandrostane-3,17-dione, m.p. 232–233° dec., red melt, $[\alpha]_D$ −140 ± 2°, ΔM_D −457°.

Anal. Calcd. for $C_{19}H_{26}O_3S_2$: C, 62.26; H, 7.15. Found: C, 62.06, 62.00; H, 6.76, 7.00.

CHICAGO 80, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

Glycinate Complexes of Zinc and Cadmium

BY BARBARA W. LOW,[1] F. L. HIRSHFELD AND F. M. RICHARDS[1]

RECEIVED MARCH 13, 1959

Crystals of $Zn(NH_2CH_2CO_2)_2 \cdot H_2O$ and $Cd(NH_2CH_2CO_2)_2 \cdot H_2O$ are approximately isomorphous. The latter, being of higher crystallographic symmetry, has been studied in somewhat greater detail although only one projection has been analyzed. In both complexes the metal coördination is octahedral, two glycine ligands chelating with the metal in *trans* planar array while the other two coördination positions are occupied by carboxyl oxygens of neighboring glycine ligands. The amino groups and the water are fully hydrogen bonded. The difference in symmetry of the two structures is not explained.

Introduction

The marked effect of zinc ion on protein solubilities[2] suggested that a study of the coördination of this metal with amino acids would be of interest (for a review of other work in this field, see Gurd and Wilcox).[3] An X-ray crystallographic investigation of the zinc glycine complex was undertaken in an attempt to establish the nature of the metal coördination in this structure. At the same time, glycine complexes with several other metals were prepared, of which the cadmium complex proved to be crystallographically similar to the zinc complex. X-Ray diffraction photographs showed the two crystals to be nearly isomorphous. The cadmium complex, of much higher symmetry, was far the more amenable to crystallographic analysis. Accordingly, when work on the zinc complex had proceeded far enough to show the general nature of the metal coördination, attention was temporarily diverted to the cadmium complex, which offered greater promise of a more detailed structure determination.

Experimental

The zinc and cadmium complexes were both prepared in the same way. The metallic oxide (1 mole) and recrystallized glycine (2 moles) were dissolved together in boiling water. After filtration, the solution was allowed to cool slowly to room temperature. Large crystals were obtained by recrystallization from water and from water–alcohol (1:1) mixtures. Micro-Kjeldahl analyses and dry-weight determinations (air, 110°) of both complexes established the crystalline composition: $M(NH_2CH_2COO)_2 \cdot H_2O$.

Morphology and Optics.—The approximate isomorphism of the two preparations was immediately evident from the morphological and optical examination. Both the zinc and the cadmium glycinate monohydrate crystallize as colorless plates elongated along [010] and usually lying on (001) (see Table I for cell dimensions and space groups). Most crystals show {100}; other forms commonly observed include {110} and {1$\bar{1}$0} of the zinc complex (designated in terms of the triclinic holosymmetric class, as discussed below), {210} and {110} for the cadmium complex. The crystals frequently show etched lines parallel to [010] on the main face (001) with principal cleavage (100). Crystals of the zinc complex tend to grow radially in clumps so that single crystals with unbroken ends are somewhat rare. In well-developed specimens both like and unlike termination has been observed.

TABLE I

CRYSTAL LATTICE CONSTANTS

Lattice dimensions measured on a General Electric Spectrogoniometer fitted with a Eulerian cradle, Goniostat,[a] for single-crystal studies. Copper radiation employed: λ $K\alpha_1$ = 1.54051, λ $K\alpha_2$ = 1.54433.

	Cadmium glycinate monohydrate	Zinc glycinate monohydrate
a, Å.	14.862 ± 0.005	15.051 ± 0.006
b, Å.	5.297 ± .005	10.441 ± .008
c, Å.	10.006 ± .003	19.692 ± .012
α, degrees		89.89 ± .05
β, degrees	90.40 ± .03	87.15 ± .05
γ, degrees		90.39 ± .05
Space group	$I2/a$	Al or $A\bar{1}$
Z	4	16
Density (calcd.), g./ml.	2.350 ± 0.004	1.990 ± 0.004
Density (flotation), g./ml.	2.338 ± 0.010	1.993 ± 0.005

[a] T. Furnas and D. Harker, *Rev. Sci. Instr.*, **26**, 449 (1955).

Viewed normally to the (001) face, crystals of both complexes give a slightly off-center optic-axis figure. They show high positive birefringence, with $\beta \| b$ and γ parallel to the trace of (101) for the zinc complex, (201) for the cadmium complex, in the plane perpendicular to b. Measurements on the zinc complex gave

$\alpha = 1.510 \pm 0.003$, $\beta = 1.547 \pm 0.003$, $\gamma = 1.648 \pm 0.003$, $2V = 67°$

Cadmium Glycinate Monohydrate.—The cadmium complex is monoclinic, with four molecules of cadmium glycinate and four of water in a cell

(1) Part of this work was carried out while (B.W.L., and F.M.R.) were members of the Department of Biophysical Chemistry, Harvard Medical School.

(2) E. J. Cohn, F. R. N. Gurd, D. M. Surgenor, B. A. Barnes, R. K. Brown, G. Derouaux, J. M. Gillespie, F. W. Kahnt, W. F. Lever, C. H. Liu, D. Mittelman, R. F. Mouton, K. Schmid and E. A. Uroma, THIS JOURNAL, **72**, 465 (1950).

(3) F. R. N. Gurd and P. E. Wilcox, *Advances in Protein Chem.*, **11**, 311 (1956).

having the dimensions listed in Table I. The crystal axes have been chosen so as to facilitate comparison of this structure with that of the zinc complex although this has resulted in unconventional representations of both unit cells. The chosen axes have the incidental advantage of being nearly orthogonal. For the cadmium complex, transformation to axes $a' = a + c$, $b' = b$,$c' = -c$ gives a more conventional cell with space group Cc or C2/c. As represented here, however, general hkl reflections occur only for $h + k + l$ even and $h0l$ reflections only for h and l even, indicating space group Ia or I2/a.

Because the cadmium atom heavily dominates the X-ray scattering intensity statistics were unsuitable as a test for a center of symmetry. It was noted, however, that reflections with h odd were generally weak, being scarcely observable except at fairly low angles, while those with h even appeared more or less uniformly strong, at least to the limit of the Cu K_α sphere. This observation suggested that the cadmium atoms were aligned in rows at intervals of $a/2$ so that they contributed maximally to structure factors with h even but not at all to those with h odd. Such an arrangement required that the cadmium be located on the a-glide plane, which could occur purely by chance if the space group were Ia but would be far less fortuitous if it were I2/a. For in the latter case, the cadmium would necessarily occupy a special position, either on the twofold axis or at a center of symmetry, and the only symmetry centers present lie on the a- or the c-glide planes. Accordingly, it was considered very likely that the space group was I2/a and, further, that the cadmium occupied a center of symmetry. The latter conclusion immediately implied that the metal coördination was planar and had a *trans* configuration.

On the assumption that the cell was centrosymmetric, it was possible to compute the b-axis electron-density projection directly, for the $h0l$ zone contained terms with even h only, and these could safely be assumed to have their signs determined completely by the cadmium contributions, which could be taken as all positive. The Fourier map computed in this way showed all the atoms except hydrogens clearly resolved and served to confirm the centric space group. Severe series-termination errors, however, seriously falsified the peak densities and, presumably, the positions of the atomic maxima. It was therefore decided to compute a difference-density projection with the cadmium peak removed. For this purpose the average amplitude of the $h0l$ spectra was plotted against scattering angle; the resulting curve agreed closely, except at low angles, with the Thomas–Fermi scattering curve for neutral cadmium[4] uncorrected for thermal motion. Accordingly, this Thomas–Fermi curve was used for the derivation of the absolute scale of the observed structure factors and for the evaluation of the cadmium contributions. This information permitted the computation of the difference-density projection (Fig. 1), which yielded the x and z-coördinates of all carbon, nitrogen and oxygen atoms and also

(4) "International Tables for the Determination of Crystal Structures, 1935," Borntraeger, Berlin.

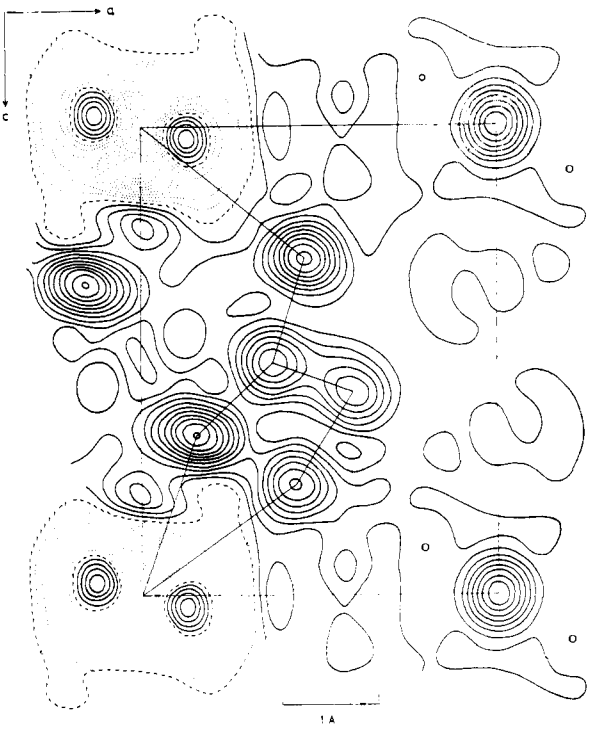

Fig. 1.—Cadmium glycinate monohydrate: difference density projected on (010). Contours at $1eA^{-2}$ intervals, zero contour broken, negative contours dotted.

revealed a very marked anisotropy in the cadmium peak. With the aid of packing models, based on the molecular dimensions found in α-glycine,[5] a thoroughly reasonable three-dimensional structure was readily fitted to the b-axis projection.

Examination of this structure indicated that any other projection would be too poorly resolved to provide much further information. Since a three-dimensional study seemed unwarranted, the investigation was terminated at this stage despite the incompleteness of the results obtained. Nevertheless, a fairly satisfactory description of the crystal structure has been achieved, whose principal features may be accepted with some confidence. This confidence rests largely on the reasonableness of the intermolecular contacts obtained but also, to some extent, on the similarity of this structure to that deduced from Patterson projections of the zinc complex (see below). Such considerations fix the probable y-coördinates of the glycine atoms to within about 0.2 Å., with a somewhat greater uncertainty in the water position. The atomic coördinates are listed in Table II.

TABLE II

ATOMIC COÖRDINATES FOR CADMIUM GLYCINATE MONOHYDRATE

	x (Å.)	y (Å.)	z (Å.)
Cd	0	0	0
C_1	1.37	1.7	2.52
C_2	2.21	3.0	2.85
N	1.61	3.9	3.83
O_1	0.58	1.2	3.31
O_2	1.70	1.1	1.43
O_3	3.713	2.6	0

(5) R. E. Marsh, *Acta Cryst.*, **10**, 814 (1957)

A prominent feature of this structure is the occurrence of extended sheets of cadmium glycinate parallel to (100), held together in two dimensions by a network of coördination bonds and hydrogen bonds (Fig. 2). These sheets are composed of

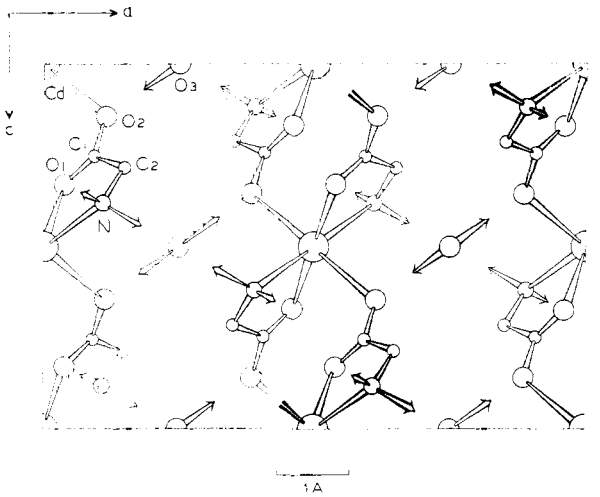

Fig. 2.—Cadmium glycinate monohydrate: unit cell contents projected on (010). Arrows indicate hydrogen bonds.

approximately planar chelate units formed by fourfold coordination of the cadmium by one nitrogen and one oxygen atom of each of two glycine ligands. The Cd–N and Cd–O bonds appear to be about 2.3 Å. long, and the N–Cd–O angle in the chelate ring is about 80° or less. At approximately right angles to the plane of these chelate rings, the free carboxyl oxygens of two neighboring glycine ligands approach the cadmium at a distance of about 2.5 Å., completing a distorted coördination octahedron similar to that apparently present in copper *d,l*-α-aminobutyrate.[6] This metal coördination would appear sufficient to hold the structure together in the *b* and *c* directions; it is reinforced by a system of hydrogen bonds that link the glycine ligands in chains parallel to *b*, with the nitrogen of each ligand hydrogen-bonded to a carboxyl oxygen of the corresponding ligand in the adjacent unit cell.

Interleaved between successive sheets of cadmium glycinate are layers of water, through each molecule of which hydrogen bonds link together four glycinate ligands in the two adjoining sheets. The water molecule donates two hydrogen bonds to neighboring carboxyl oxygen atoms and accepts two bonds from neighboring amide groups. Thus all hydrogen atoms available for hydrogen bonding are so engaged, and this bonding helps to produce a highly cohesive structure in all directions. The observed cleavage parallel to (100) presumably involves rupture of the hydrogen bonds to and from the water.

Little information has been obtained about the shape of the glycine ligand beyond the observation that it appears to be approximately planar. The nitrogen atom seems, however, to deviate somewhat from the mean plane, perhaps under the influence of the hydrogen bond linking it to the adjacent ligand in the chain. The C_1–O_1 bond, perhaps unexpectedly, appears somewhat shorter than the C_1–O_2 bond.

The observed anisotropy of the cadmium atom is in accord with the supposition that it is held most strongly by the four chelating bonds, vibrating with the greatest amplitude in a direction approximately normal to their plane, where the coördination bonds appear to be longer.

Zinc Glycinate Monohydrate.—The zinc complex is triclinic, pseudomonoclinic and has been referred for the sake of comparison with the cadmium complex to a non-primitive cell containing sixteen molecules whose dimensions are given in Table I. This cell is evidently similar to that of the cadmium complex except for a doubling of the *b*- and *c*-axes. For the primitive cell, containing eight molecules

$a' = a = 15.051$, $b' = b = 10.441$, $c' = -(b + c)/2 = 11.153$ Å., $\alpha' = 118.02°$, $\beta' = 92.51°$, $\gamma' = 90.39°$

In addition to centering on the A face, indicated by the absence of reflections with $k+l$ odd, the crystal shows a high order of pseudosymmetry that tends to simulate some of the genuine symmetry of the cadmium complex. For example, reflections with $h+(k-l)/2$ odd are systematically weak, in apparent mimicry of the body centering of the cadmium structure. Further, the *h*0*l* and, to a lesser degree, the *h*1*l* spectra are conspicuously weaker when *h* is odd than when it is even, and in addition, the 0*kl* and $0\bar{k}l$ intensities are about equal when *k* and *l* are even.

The crystals were tested for piezoelectricity with negative results. On this evidence and on the further grounds that the zinc molecule was, by analogy with the cadmium complex, almost certainly centrosymmetric and therefore unlikely to occur in a non-centric cell,[7] the space group was assumed to be A1.

In view of the complexity of the crystal structure, progress toward its solution was sought through an approximation in which some of the pseudosymmetry of the actual structure was treated as though it were genuine symmetry. Thus, the reflections with $h+(k-l)/2$ odd were entirely neglected so that one might deal, in effect, with a subcell containing only four units of zinc glycinate monohydrate, of which two comprised the simplified asymmetric unit. Patterson projections of this subcell were computed (Fig. 3a and b) along the *a* and *b*-axes, the *b*-axis projection being further simplified by neglect of *h*0*l* reflections with odd *h*.

The *a*-axis projection showed a pair of sharp peaks near the position $y = {}^1/_2, z = 0$. These were interpreted as representing multiple Zn–Zn vectors arising from the pairwise overlap of the four zinc atoms at the positions $(y, z) = \pm (0.244, 0.015)$. Once the centers of the zinc glycinate molecules had been located, the molecular orientations were readily deduced from the distribution of secondary peaks in the Patterson map. Interpretation of this map was completed with the identification of the zinc–water vectors. However, because of the molecular overlap in this projection, the structure

(6) A. J. Stosick, THIS JOURNAL, **67**, 365 (1945).

(7) F. H. Herbstein and F. R. L. Schoening, *Acta Cryst.*, **10**, 657 (1957).

derived therefrom remained subject to an ambiguity that was only resolved by comparison with the structure subsequently found for the cadmium complex.

In the *b*-axis Patterson projection a prominent ridge along the trace of (404), corresponding, as might be expected, with the direction of highest refractive index (see above), helped to fix the molecular orientation while strongly implying that the molecule was wholly or nearly planar. A pronounced elongation of the origin peak at right angles to this ridge, interpreted in conjunction with the evidence from the *a*-axis projection, gave the approximate *x*-coördinates of the four zinc atoms as ± 0.024 and ± 0.524. These simplified Patterson projections led to a broad outline of the structure that was adequate to establish the essentially octahedral coördination about the zinc. The details of this structure became somewhat clearer through subsequent comparison with the similar structure of the cadmium complex described above. The principal difference between the two is that the zinc complex apparently has the chains of molecules along the *b*-direction staggered, with alternate molecules displaced by about 0.4 Å. from the chain axis in a direction approximately normal to the mean molecular plane. The reason for this staggered arrangement is not evident as inspection of a model of the cadmium glycinate structure suggests no obvious reason why replacement of cadmium by zinc should cause a lowering of the symmetry. Moreover, it appears impossible to decide, without a full three-dimensional study of this most unpromising structure, just how the actual arrangement differs from that in the approximate model considered above. Accordingly, one cannot say at present what is the basic difference in crystal packing between the two complexes. The same hydrogen bonds may be assumed to occur in the two structures and similar coördination of the metal to the organic ligand. But there is no evidence whether, for example, the coördination octahedron is less symmetric than that of the cadmium, or even whether the four crystallographically non-equivalent zinc atoms show identical coördination. It is quite consistent with the available evidence that there may be four distinct types of coördination octahedra with different kinds and degrees of asymmetry. The present study clearly leaves unanswered questions at least as interesting as those it has resolved.

Discussion

Some tentative conclusions may be offered with regard to the coördination found in the cadmium complex, to which that in the zinc complex must be somewhat similar. The observed coördination is essentially sixfold, with four coplanar bonds in the chelate rings and two other bonds, apparently longer, approximately perpendicular to these. The infrared spectrum of the zinc complex suggests[8] that only the nitrogen atoms are covalently bound to the metal, through linear *sp* orbitals, while the carboxyl groups are joined to the metal primarily through electrostatic interaction. Because of the somewhat arbitrary procedure followed in deriving the present structures, they certainly do not provide a crucial test of such a formulation for either the zinc or the cadmium complex. However, the apparent asymmetry of the coördination octahedra does seem to indicate that the degree of covalency of the metal–oxygen bonds is insufficient to require complete sp^3d^2 hybridization. For where such hybridization does occur, the cadmium and zinc atoms, having completely filled inner *d* shells[9] tend to be surrounded by regular coördination octahedra as in the hexahydrates and hexamines.

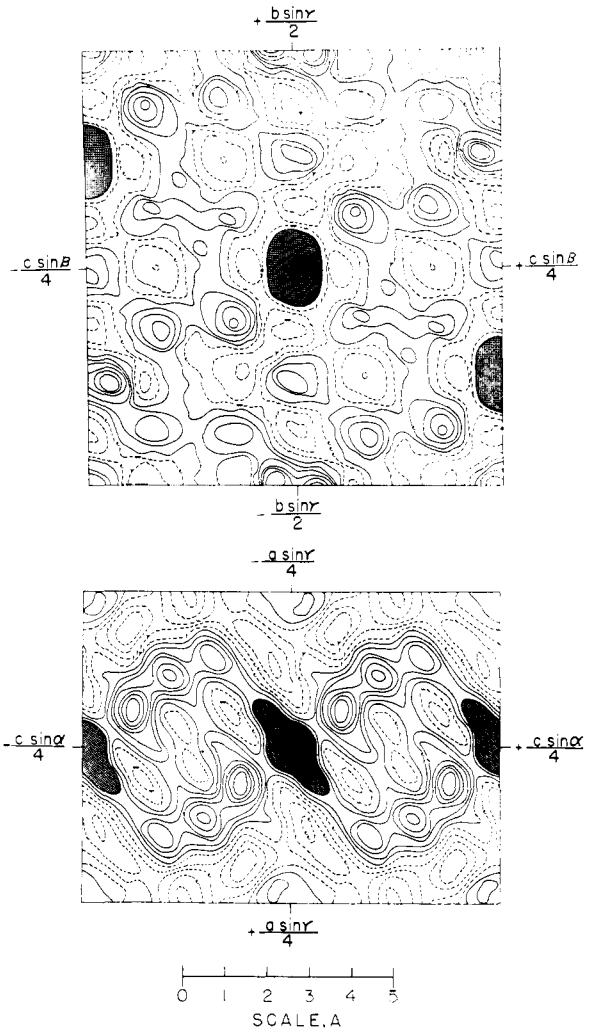

Fig. 3a, b.—Zinc glycinate monohydrate *a*- and *b*-axis Patterson projections: contours at arbitrary intervals.

A very similar situation occurs in zinc 8-hydroxyquinolinate dihydrate,[10] where two water molecules are coördinated to the zinc by longer bonds than those found in the chelate rings. The authors of that study have put forward an explanation for the distortion of the N–Zn–O angles in the chelate rings which may be applicable to the present structures as well. But no reason for the 2.27 Å.

(8) D. M. Sweeny, C. Curran and J. V. Quagliano, THIS JOURNAL, **77**, 5508 (1955).

(9) J. R. Gillespie and R. S. Nyholm, *Quart. Rev.*, **11**, 339 (1957).

(10) L. L. Merritt, Jr., R. T. Cady and B. W. Mundy, *Acta Cryst.*, **7**, 473 (1954).

length of the zinc–water bond has been given. Nor has it been explained why the coördination octahedron in zinc aspartate trihydrate[11] should contain five Zn–O bonds ranging in length from 2.08 to 2.21 Å. It is evident that much more work is needed on complexes of this type, and on coördination compounds in general, before the nature of the metal–donor bond is clearly understood. The authors do not intend to continue this work.

Acknowledgments.—This work was supported, at Harvard University, by the Eugene Higgins Trust, by grants from the Rockefeller Foundation and the National Institutes of Health, by contributions from industry and by funds of Harvard University. The work at Harvard was carried out while one of us (F.M.R.) was an Atomic Energy Commission Predoctoral Research Fellow in the Biological Sciences.

This investigation, at Columbia University, was supported by a reasearch grant, A-1320(C), from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

The authors wish to acknowledge with gratitude the help and assistance of Dr. S. C. Abrahams, who tested the zinc glycinate monohydrate crystals for piezoelectricity, of Dr. F. R. N. Gurd and Dr. P. E. Wilcox, who supplied some zinc glycinate crystals, and of Miss Marion Smith, who performed micro-Kjeldahl analyses of both preparations.

(11) T. Doyne and R. Pepinsky, *Acta Cryst.*, **10**, 438 (1957).

NEW YORK, N. Y.

[CONTRIBUTION FROM THE BIOCHEMICAL RESEARCH LABORATORY, THE DOW CHEMICAL COMPANY]

Studies on the Enzyme Dextransucrase. IV. Altering the Substrate Specificity Pattern of the Enzyme

BY W. BROCK NEELY

RECEIVED FEBRUARY 23, 1959

Michaelis constants and maximum initial velocities were determined for dextransucrase from 5 to 35°. The sharp break in the Michaelis-temperature plot at 30° is explained on the basis of a reversible denaturation. The mild denaturation so alters the specificity site of the enzyme that maltose can act as a donor of glucosyl groups in addition to the normal substrate of sucrose.

Dextransucrase acting on sucrose in the presence of a suitable acceptor causes a transfer of the glucosyl group to the acceptor with the ultimate formation of the polysaccharide dextran. This enzyme has received a great deal of attention by many workers since it was first demonstrated that cell free extracts of *Leuconostoc mesenteroides* were able to catalyse this particular reaction.[1,2] Dextransucrase preparations are obtained readily and some of the properties of this transferring enzyme have been described.[1-9] In addition, the role of the acceptor molecule has been extensively studied[10-14] and certain structural requirements necessary for this particular activity are slowly being evolved. However, the characteristics which endow sucrose with the ability to act as a practical source of glucosyl groups have been neglected. This communication is concerned with the rigidity in the requirements of the enzyme for the substrate sucrose. The kinetics of the enzyme were studied in the temperature range 5–35°. The interpretation of the rate data is based on a reversible denaturation of the enzyme with subsequent loss of substrate specificity. The ability of the partially denatured enzyme to utilize maltose as an alternate glucosyl donor substantiates the proposal that the enzyme did indeed lose part of its high selectivity for the substrate sucrose.

(1) E. J. Hehre, *Science*, **93**, 237 (1941).
(2) E. J. Hehre and J. Y. Sugg, *J. Exptl. Med.*, **75**, 339 (1942).
(3) H. J. Koepsell and H. M. Tsuchiya, *J. Bacteriol.*, **63**, 293 (1952).
(4) E. J. Hehre, *J. Biol. Chem.*, **163**, 221 (1946).
(5) W. B. Neely, THIS JOURNAL, **80**, 2010 (1958).
(6) W. B. Neely, *Nature*, **182**, 1007 (1958).
(7) W. B. Neely, *Arch. Biochem. Biophys.*, **79**, 297 (1959).
(8) R. W. Bailey, S. A. Barker, E. J. Bourne and M. Stacey, *J. Chem. Soc.*, 3530 (1957).
(9) R. W. Bailey, S. A. Barker, E. J. Bourne and M. Stacey, *ibid.*, 3536 (1957).
(10) H. J. Koepsell, H. M. Tsuchiya, N. M. Hellman, A. Kazenko, C. A. Hoffman, E. S. Sharpe and R. W. Jackson, *J. Biol. Chem.*, **200**, 793 (1952).
(11) R. W. Bailey, S. A. Barker, E. J. Bourne and M. Stacey, *Nature*, **176**, 1164 (1955).
(12) S. A. Barker, E. J. Bourne, P. M. Grant and M. Stacey, *J. Chem. Soc.*, 601 (1958).
(13) W. B. Neely, *Arch. Biochem. Biophys.*, **79**, 154 (1959).
(14) C. S. Stringer and H. M. Tsuchiya, THIS JOURNAL, **80**, 6620 (1958).

Experimental

Enzyme Production.—The isolation of a crude cell-free dextransucrase solution from *Leuconostoc mesenteroides* NRRL B-512F was based on the procedure of Tsuchiya and co-workers.[15] The assay used was described by Tsuchiya, Koepsell and co-workers[3,15] where the fructose liberated was measured by the Somogyi method[16] as modified by Nelson.[17]

Kinetics.—Kinetic measurements were conducted as described previously[5] with the following modifications, the buffer used was 0.05 *M* acetate *p*H 5.2 and the length of incubation was 0.5 hr. Michaelis constants and maximum initial velocities were determined by the graphical method of Lineweaver and Burk.[18]

Urea Denaturation.—Solutions of enzyme (5 ml.), urea and 0.05 *M* acetate buffer *p*H 5.2 to make a total volume of 25 ml. were allowed to react for 24 hr. at 5°. At the conclusion assays were conducted in the usual manner on the various solutions ranging from 0–8 *M* urea. It was determined that the highest concentration of urea had no effect on the reducing sugar analysis. The control enyzme (no urea) and the enzyme treated with 8 *M* urea were then dialyzed against several changes of a 0.05 *M* citric acid buffer *p*H 5.2 for 24 hr. at 5°. Assays were repeated on the dialyzed samples.

(15) H. M. Tsuchiya, H. J. Koepsell, J. Corman, G. Bryant, M. O. Bogard, V. H. Feger and R. W. Jackson, *J. Bacteriol.*, **64**, 521 (1952).
(16) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).
(17) N. Nelson, *ibid.*, **153**, 375 (1944).
(18) H. Lineweaver and D. Burk, THIS JOURNAL, **56**, 658 (1934).