morphological complexity. These are *Navicula incerta, Nitzschia angularis, Nitzschia thermalis* (thermophile), *Nitzschia alba* (colorless), *Cylindrotheca fusiformis, Cyclotella cryptica,* and *Phaeodactylum tricornutum* (fusiformis and oval) (*18*).

The occurrence of 3,4-dihydroxyproline in the proteinaceous components of the diatom wall suggests that the new amino acid may be a determinant of the molecular structure of the organic matrix associated with silicification, as are hydroxyprolines in collagen.

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2,3-cis-3,4-trans-3,4-Dihydroxy-L-proline: Mass Spectrometry and X-ray Analysis

Abstract. *Mass spectrometry showed the new amino acid from diatom cell walls to be a dihydroxyproline. X-ray analysis of the unmarked free amino acid by the use of the symbolic addition procedure revealed the complete three-dimensional structure as 2,3-*cis*-3,4-*trans*-3,4-dihydroxy-L-proline with carbon number 3 being 0.06 angstrom above the plane of the pyrrolidine ring.*

The preceding report (*1*) describes the isolation of a new amino acid from the cell walls of various diatoms. On the basis of nuclear magnetic resonance (NMR) spectrometry, mass spectrometry, and complete x-ray analysis, the structure of 2,3-*cis*,3,4-*trans*-3,4-dihydroxy-L-proline has been proposed.

The mass spectra of a synthetic mixture of the two *cis*-3,4-glycols obtained by permanganate oxidation of 3,4-dehydro-DL-proline (*2*) and of the new natural amino acid (*1*) were compared on a Hitachi RMU-6D mass spectrometer at 70 ev and 350° to 400°C. The spectra were virtually identical. Both compounds exhibited a large $M+1$ peak at m/e (mass to charge) = 148. The exact mass of this peak for the naturally occurring amino acid confirmed its identity as a 3,4-dihydroxyproline. Fragmentation of both compounds, by loss of the carboxyl group, led to a base peak at $m/e = 102$. Tentative formulations for the other major fragments at $m/e = 69$ and 87 are given in Fig. 1.

A single crystal of the new amino acid was subjected to an x-ray diffraction analysis. A total of 611 independent data were collected with Cu radia-

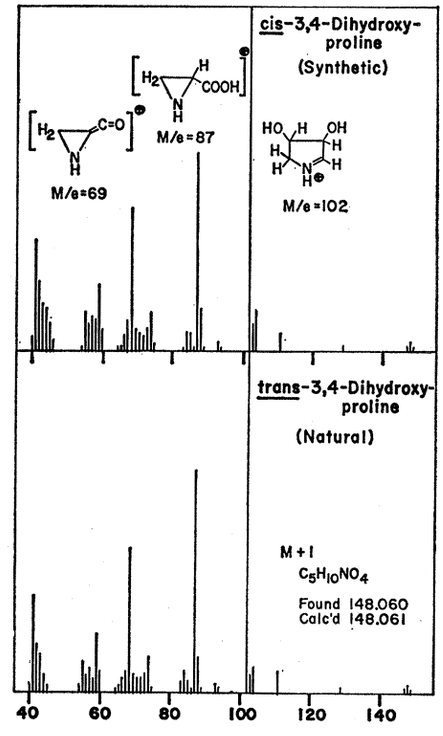


Fig. 1. Comparison of the mass spectra of the *cis*-glycols prepared from 3,4-dehydro-DL-proline by oxidation with permanganate (*1*) and of the *trans*-glycol of L-proline from diatom cell walls.

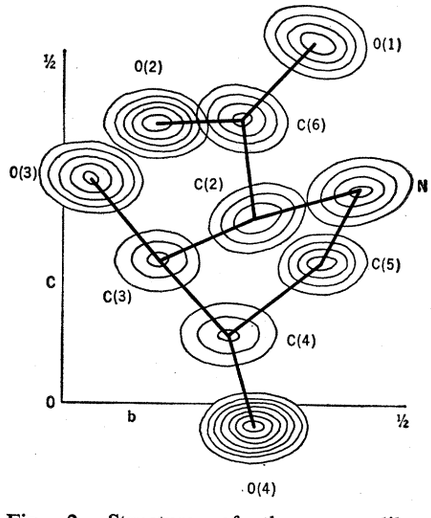


Fig. 2. Structure of the new dihydroxyproline from diatoms as determined by taking sections through maximum densities of the initial three-dimensional E-map computed with the phases directly derived from the intensities.

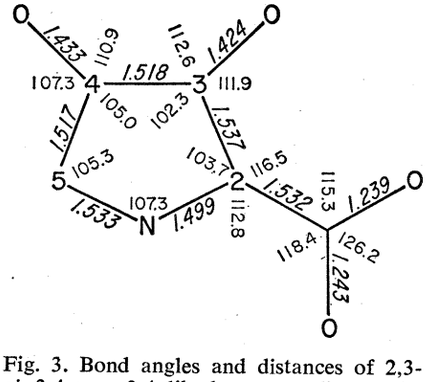


Fig. 3. Bond angles and distances of 2,3-*cis*-3,4-*trans*-3,4-dihydroxy-L-proline, the new natural amino acid from diatoms.

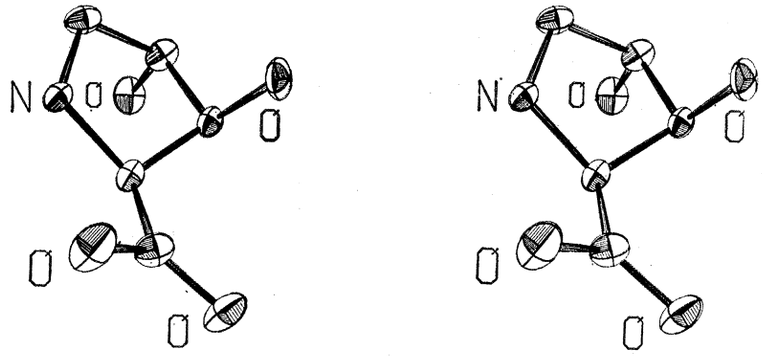
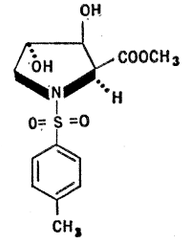


Fig. 4. Computer-made stereodrawing of the configuration of natural 2,3-*cis*-3,4-*trans*-dihydroxy-L-proline as determined by x-ray analysis. The picture should be seen with a three-dimensional viewer for printed stereophotographs (commercially available, Stereo-Magniscope, Inc., Elmhurst, N.Y.).

tion from an acicular prism (0.06 by 0.06 mm cross section) with the multiple-film, equi-inclination Weissenberg technique. Although long exposures were required, because the crystal was very small, the scattering extended to the edge of the Cu sphere. The space group is orthorhombic, $P2_12_12_1$, with cell parameters $a = 8.38$ Å, $b = 8.43$ Å, $c = 8.57$ Å; there are four molecules in the unit cell. The symbolic addition procedure (*3*) for noncentrosymmetric crystals was used to determine the phases of the strong and moderately strong reflections. The initial E-map computed with 214 terms with $|E| > 1.0$ for which phases had been determined revealed the structure of the molecule (Fig. 2). Hydrogen atoms were located in a difference map and the least-squares refinement resulted in an R-factor of 5.8 percent.

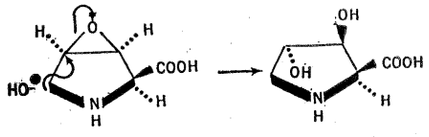
The x-ray diffraction results confirm the structural formula and establish the conformation of the molecule. The bond distances and angles are shown in Fig. 3. Atoms C(4), C(5), N, and C(2) of the five-membered ring lie in a plane, to within ± 0.009 Å, whereas C(3) is 0.60 Å above the plane. This conformation differs from that of L-proline (*4*) and of natural (*trans*) 4-hydroxy-L-proline (*5*), where it is atom C(4) which is out of the plane in the five-membered ring. The carboxyl group is equatorial while each of the two hydroxyl groups is axial to the ring. The N atom is 0.23 Å out of the plane containing the carboxyl group and the α-carbon (Fig. 4). The molecule exists as a zwitterion with an extra proton on the N atom and a negative charge on the carboxyl group. Four different hydrogen bonds, two NH · · · O and two OH · · · O, bind the molecules in the crystal into a tight network, resulting in low thermal factors for the individual atoms.

The closest synthetic analog of the new amino acid is the racemic *trans*-



I

glycol (I) prepared from *N*-tosyl-3,4-dehydro-DL-proline methyl ester (*6*). Such *trans*-glycols (III) are accessible by ring opening of the *cis*-epoxide (II) (*5*). In view of the recent isolation of (arene)oxide intermediates in microsomal metabolism of (aromatic) substrates (*7*) and the existence of a special oxide hydrase (*8*), such a bio-



II III

genetic pathway may now have to be considered, although the biosynthesis of hydroxyproline in collagen (*9*) by proline hydroxylase (*10*) certainly does not involve a dehydroproline precursor (*11*). The new amino acid bears a formal relationship to the antibiotic anisomycin (*12*).

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7 March 1969

Venom Neutralization by Rattlesnake Serum Albumin

Abstract. *The blood serum of the eastern diamond back rattlesnake* (Crotalus adamanteus) *neutralizes lethal doses of* C. adamanteus *venom in mice. The protective capacity of the serum is associated with the serum albumin, rather than the immunoglobulin fraction of the blood. Neither the serum nor its albumin fraction form precipitin bands on immunophoresis against venom.*

Blood serums of several snakes neutralize the venom of other snakes both in vitro and in vivo (*1*). Useful quantitative data on the effectiveness of specific serums are incomplete or entirely lacking, however. Although commercially available horse serum antivenins function as globulin centered antibodies to venom antigens (*2*), there have been no indications that this is the mechanism in venom neutralization by snake serum. As a result of our investigation into the ability of blood serum and serum fractions of the eastern diamond back rattlesnake (*Crotalus adamanteus*) to protect mice from otherwise lethal doses of *C. adamanteus* venom, we can now report some quantitative data on dosage effectiveness. In addition, we have found that the high degree of protection observed is not due to antibody-antigen interactions and is associated with the albumin rather than globulin fraction of the serum.

Crotalus adamanteus venom was ob-