precision optics were employed (Corning, No. 1½).

Much of the tendency of slides to deteriorate over a period of a few hours was traced to evaporation through pinhole imperfections in the wax used to seal the preparation. This was eliminated by using two cover slips of unequal size creating a shoulder on which the wax could bind, and by using a mixture of one part Vaseline to three parts paraffin instead of pure paraffin.

Although the effect of each of the above refinements is small, they combine to permit recording from primate cones lying on their sides. No changes were necessary in the electronics.

Several hundred foveal and parafoveal primate cones (mostly human) have been examined oriented at right angles to the measuring beam in the course of developing these refined techniques. Ratios of signal to noise have been very poor until the series shown in Fig. 2; these are the best records which have been obtained so far and demonstrate the feasibility of the technique.

Each receptor is about 0.8 μ in diameter and absorbs about 1.3 percent at maximum. The pigment density in primate rods and cones is similar and is in turn comparable to the densities (*1, 3*) for frog rods and fish cones. With a specific absorption of 1.8 percent/μ, the total absorption of a foveal cone 45 μ in length would be about 50 percent. This probably represents a lower limit due to light scattered around the receptor. Psychophysical experiments (*10, 11*) have indicated that the maximum absorption may be in excess of 80 or 90 percent. However, too few successful preparations were obtained to determine the relative numbers of cones of each spectral class, or to confirm earlier measurements (*4, 5*) of the maximum absorption of each class.

W. H. DOBELLE,* W. B. MARKS
E. F. MACNICHOL, JR.†
Thomas C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, Maryland

References and Notes

1. W. B. Marks, thesis, Johns Hopkins University (1963).
2. P. A. Liebman, *J. Opt. Soc. Amer.* **54**, 1451 (1964).
3. W. B. Marks, *J. Physiol.* **178**, 14 (1965).
4. ——— W. H. Dobelle, E. F. MacNichol, Jr., *Science* **143**, 1181 (1964).
5. P. K. Brown and G. Wald, *Science* **144**, 45 (1964).
6. W. H. Dobelle, thesis, Johns Hopkins University (1967).
7. The signal-to-noise ratio is approximately equal to the height times the square root of the area. Therefore, $\frac{\text{end-on}}{\text{side}} \approx \frac{30\,(0.8)^{\frac{1}{2}}}{0.8\,(10)^{\frac{1}{2}}} \approx$ tenfold decrease in the ratio of the signal to noise.
8. The objectives were furnished by Zeiss, Leitz, Zeiss-Jena, Wild, Bausch and Lomb, American Optical, and Nikon.
9. Samples of all embedding media were originally supplied by Sunkist Laboratories, Box 2706, Los Angeles, California 90054. The gelatin is no longer available. However, extremely transparent 300 Bloom (a measure of viscosity) gelatin is presently produced by Swift and Co., 1211 W. 22nd St., Oak Brook, Illinois 60521.
10. G. Brindley, *Proc. Phys. Soc.* (*London*) **68B**, 862 (1955).
11. P. L. Walraven, thesis, University of Utrecht (Institution for Perception RVO-TNO) Soesterberg, Netherlands (1962).
12. Supported by NSF grant GB-2705 and NIH grant NB-03582. Direct reprint requests to Building 512, University of Utah, Salt Lake City 84112.
* Present address: Joint program of Departments of Physiology and Computer Sciences and Division of Artificial Organs, University of Utah, Salt Lake City 84112.
† Present address: National Institute of Neurological Diseases and Stroke, Bethesda, Maryland 20014.

23 July 1966; revised 22 August 1969

Imidodiphosphate and Pyrophosphate: Possible Biological Significance of Similar Structures

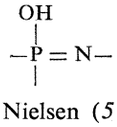
Abstract. *The structure of sodium imidodiphosphate has been determined by single crystal x-ray diffraction. The P–N–P bond angle (127.2°) and P–N bond distance (1.68 angstroms) are remarkably similar to newly refined values for the P–O–P bond angle (128.6°) and the bridging P–O bond distance (1.63 angstroms) of sodium pyrophosphate. This close similarity may explain why P–N–P linkages in algal "polyphosphates" escaped detection until recently and why adenosine triphosphate analogs with this linkage mimic adenosine triphosphate so closely.*

Correll (*1*) has found imidodiphosphate (P–N–P) linkages in algal "polyphosphate." This is the first instance of P–N–P linkages being observed in a natural product. Almost nothing is known of the metabolism of P–N–P compounds, but knowledge of the molecular dimensions of the P–N–P linkage should aid in these studies. In addition, an adenosine triphosphate (ATP) analog in which an imidodiphosphate grouping replaces the terminal pyrophosphate has been synthesized (*2*). Correll's finding and the striking similarity of this analog to ATP in binding to contractile proteins (*2*) led us to investigate the crystal and molecular structure of sodium imidodiphosphate in relation to that of sodium pyrophosphate.

Crystals of tetrasodium imidodiphosphate·decahydrate were prepared according to Nielsen *et al.* (*3*). The crystals were monoclinic, with space group $C2/c$ and cell dimensions $a = 17.069$, $b = 6.905$, and $c = 14.752$ Å, and $\beta = 110.33°$, and were isomorphous with tetrasodium pyrophosphate·decahydrate. The measured density of 1.80 g/cm^3 and the calculated density of 1.812 g/cm^3 indicated that there were four molecules per unit cell. Of the 1065 intensities measured with a Picker diffractometer, 985 were observed above background. The positional parameters and anisotropic thermal parameters were refined by least squares methods (*4*) to an R-index

$$R = \frac{\Sigma\,||F_o| - |F_c||}{\Sigma\,|F_o|}$$

of 0.054. All hydrogen atoms were located on the final electron density map but were not included in the refinement (Table 1; Fig. 1a). There was no evidence for the tautomeric form



as suggested by Nielsen (*5*). However, the shortened P–N bond distances, 1.68 Å versus 1.77 Å for the single P–N bond distance in $NaPO_3NH_3$ (*6*), indicates at least partial double bond character of the P–N–P linkages.

Table 1. Comparison of the geometries of sodium pyrophosphate, sodium imidodiphosphate, and methylene diphosphonic acid. The standard deviation on the least significant digit is given in parentheses.

Bond	$O(PO_3)_2NA_4 \cdot (H_2O)_{10}$*	$O(PO_3)_2Na_4 \cdot (H_2O)_{10}$	$HN(PO_3)_2Na_4 \cdot (H_2O)_{10}$	$CH_2(PO_3)_2H_4$†
P–X	1.63Å	1.631(7)Å	1.678(5)Å	1.79Å
P–O (ave)	1.48Å	1.512(20)Å	1.521(7)Å	1.54Å
P–P		2.942(16)Å	3.006(3)Å	3.05Å
P–X–P	133.80°	128.7(3)°	127.2(5)°	117°

* Calculated from 246 observed structure factors using zonal data refinement ($R = 0.22$) (*7*).
† See (*15*).

For comparison purposes, structural data for sodium pyrophosphate (*7*) are given in Table 1. Because of the high precision of the imidodiphosphate dimensions, the original 1023 F_o from the three-dimensional film data for pyrophosphate were used in a refinement of the positional and anisotropic thermal parameters of the atoms. A final value of $R=0.136$ was obtained, although the thermal parameters did not remain positive definite on four atoms. This refinement markedly changed the P–O–P bond angle (134° to 129°) and increased the nonbridge P–O bond distances slightly (1.48 to 1.51 Å) (Table 1, column 2; Fig. 1b).

For further comparison, the structural data for the analogous carbon compound, methylenediphosphonic acid, are given in Table 1, column 4. These data are of interest since ATP, adenosine diphosphate (ADP), and guanosine triphosphate (GTP) analogs containing P–C–P linkages have been studied extensively (*8, 9*).

Structurally, the important differences between these three compounds is centered around the P–X–P linkage. The P–X bond length increases systematically from P–O (1.63 Å) to P–N (1.68 Å) to P–C (1.79 Å), while the P–P distance increases only slightly, 2.94, 3.00, and 3.05 Å, respectively. Of most interest are the P–X–P bond angles. The P–O–P and P–N–P bond angles are only slightly (but significantly) different, 129° and 127°, respectively. This contrasts with the smaller P–C–P angle of 117° (*10*). The combination of the longer P–C bond distance and the more acute P–C–P bond angle may explain why the methylene analog of ATP binds so weakly to myosin and actomyosin (*11*), because the methylene group with its two hydrogens protrudes to a much greater degree than does either the oxygen or imido linkage (*12*). In the imido linkage, the proton is effectively buried between the two PO_3 groups, as evidenced by its failure to hydrogen bond to the water molecules in the crystal lattice.

The overall striking similarity of imidodiphosphate and pyrophosphate structures explains to a large degree the similarity of interaction of ATP and the imidodiphosphate analog of ATP with myosin and heavy meromyosin (*2*). Further evidence of the similarity is that the imidodiphosphate ATP analog mimics ATP in relaxing muscle. (*13*). By contrast the methylene analog of ATP is without effect. The usefulness of the substitution of P–N–P bonds for P–O–P linkages in biologically important molecules then is apparent and structurally reasonable.

MARLIN LARSEN, ROGER WILLETT
Department of Chemistry,
Washington State University,
Pullman 99163

RALPH G. YOUNT
Departments of Agricultural Chemistry and *Chemistry,*
Washington State University

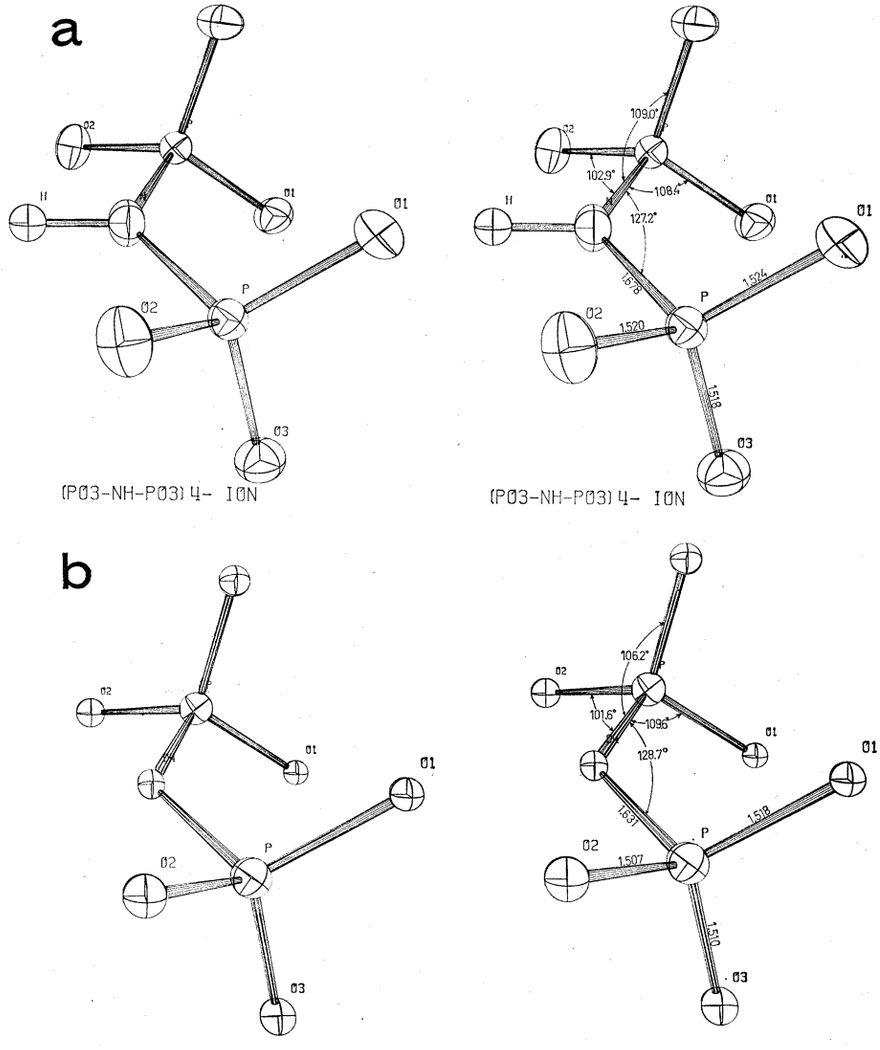


Fig. 1. (a) Stereodiagram of imidodiphosphate tetra-anion. A special viewer (*14*) should be used to see the structure in three dimensions. (b) Stereodiagram of pyrophosphate tetra-anion.

References and Notes

1. D. L. Correll, *Science* **151**, 819 (1966).
2. R. G. Yount, D. Babcock, W. Ballantyne, *Abstracts Seventh International Congress of Biochemistry* (Tokyo, Japan, 1967), p. 926; R. G. Yount, D. Ojala, D. Babcock, in preparation.
3. M. L. Neilsen, R. R. Ferguson, W. S. Coakley, *J. Amer. Chem. Soc.* **83**, 99 (1961).
4. W. R. Busing, K. O. Martin, H. A. Levy, Oak Ridge Fortran Least Squares (ORFLS), *U.S.A.E.C. Report ORNL-TM-305* (1962).
5. M. L. Nielsen, *Inorg. Chem.* **12**, 1760 (1964).
6. D. W. J. Cruickshank, *Acta Crystallogr.* **17**, 671 (1964).
7. D. M. MacArthur and C. A. Beevers, *ibid.* **10**, 428 (1957).
8. T. C. Myers, K. Nakamura, J. W. Fletcher, *J. Amer. Chem. Soc.* **85**, 3292 (1963); T. C. Myers, K. Nakamura, A. B. Danielzadeh, *J. Org. Chem.* **30**, 1517 (1965); J. W. B. Hershey and R. E. Munro, *J. Mol. Biol.* **18**, 68 (1966); L. Simon, T. C. Myers, M. Mednieks, *Biochim. Biophys. Acta* **103**, 189 (1965).
9. Miles Laboratories, Inc., Elkhart, Indiana.
10. The effect of ionizing the phosphate groups is not known but presumably would increase the P–C–P bond angle slightly.
11. C. Moos, N. R. Alpert, T. C. Myers, *Arch. Biochem. Biophys.* **88**, 183 (1960).
12. An alternate possibility is that the imido and methylene analogs of ATP form different metal ion complexes, with the imido analog complexes being the most similar to ATP. Measurement of the binding constants of the imido and methylene analogs of ATP with Mg^{2+}, Ca^{2+}, or Mn^{2+} show both analogs to be equal or better ligands than ATP (R. Yount and D. Babcock, unpublished observations).
13. R. A. Chaplain and B. Frommelt, *Kybernetik* **5**, 1 (1968).
14. Commercially available from Stereo-Magniscope, Inc., Elmhurst, New York.
15. F. M. Lovell, *Abstr. Amer. Cryst. Ass.*, p. 86 (1964).
16. Supported by an NIH predoctoral fellowship (M.L.) and by grants from NIH (A.M. 05195) and the Muscular Dystrophy Associations of America. College of Agriculture paper No. 3306, project No. 1614. We thank C. A. Beevers for providing three-dimensional film data for pyrophosphate.

7 July 1969; revised 13 August 1969