Acta Cryst. (1966). **20**, 712

Crystal Structure of the Stable Modification of 2-Aminoethylphosphonic Acid, β-Ciliatine

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(*Received* 2 *August* 1965)

The crystal structure of β-ciliatine, the stable modification of 2-aminoethylphosphonic acid, NH_3^+–CH_2–CH_2–PO_3H^-, was studied by three-dimensional single-crystal data collected by a computer-controlled diffractometer. The crystals are orthorhombic with eight formula units in a unit cell of dimensions $a=10{\cdot}35_5$, $b=10{\cdot}76_7$ and $c=8{\cdot}77_2$ Å, space group *Pbca*. The zwitterion molecule takes a *trans* configuration around the central methylene linkage. All the bond distances and angles are quite normal.

The crystal structure and the hydrogen bond system almost duplicate those of 2-aminoethanol phosphate, NH_3^+–CH_2–CH_2–OPO_3H^-, although the latter molecule possesses a *cis* configuration around the methylene linkage.

Introduction

2-Aminoethylphosphonic acid, NH_3^+—CH_2—CH_2—PO_3H^-, was first discovered by a paper-chromatographic study of sheep rumen ciliate protozoa (Horiguchi & Kandatsu, 1960) and was named ciliatine. The compound represents the first example of the occurrence of a C–P bond in biological materials. It was then found that the acid was also present in some bacteria; *e.g.* about 13·1% of the total phosphorus content of *Tetrahymena* exists as this form (Kandatsu & Horiguchi, 1962). The occurrence of the acid and its glycerol ester in the sea anemone, *Anthopleura elegantissima*, was also reported (Kitteredge, Roberts & Simonsen, 1962). The wide distribution of ciliatine in lower animals and its possible role in the metabolism prompted a crystallographic study of this compound in order to obtain its accurate molecular configuration. It is also of interest to compare the configuration and crystal structure with those of 2-aminoethanol phosphate NH_3^+–CH_2–CH_2–OPO_3H^-; the latter molecule exhibits an interesting *cis* form (Kraut, 1961).

Ciliatine is dimorphic (Horiguchi & Kandatsu, 1964); in the presence of water vapor the unstable α form transforms irreversibly into the β form. Unfortunately, it was not possible to obtain single crystals of the α form suitable for single-crystal studies. The present paper deals only with the β form and comparison between these two phases in view of the crystal structures, as well as molecular configurations, is left unanswered.

Experimental

A single crystal of β-ciliatine was ground into a sphere of approximately 0·2 mm in diameter and mounted on a General Electric Goniostat which was attached to a Picker biplane diffractometer; this diffraction equipment is part of CCXD, a computer-controlled diffractometer, operated under a stored program in an IBM 1620 computer (Cole, Okaya & Chambers, 1963). The unit-cell dimensions and space group were determined with this specimen (Cu $K\alpha$); $a=10{\cdot}35_5$, $b=10{\cdot}76_7$, $c=8{\cdot}77_2$ Å, space group *Pbca* with 8 formula units per unit cell. ($\varrho_{\text{obs}}=1{\cdot}700$ g.cm^{-3}, $\varrho_{\text{calc}}=1{\cdot}697$ g.cm^{-3}.) Integrated intensities were obtained from the specimen by using Cu $K\alpha$ radiation. For each reflection, the orientation of the crystal was first tested by optimizing the ω and χ settings; the intensity data were then recorded by step-scanning around the 2θ axis. The intervals between steps were varied according to the 2θ values at which the reflections occurred. In the course of optimizing the ω setting of a reflection, the maximum and minimum counts were recorded, and if the difference between these two counts was less than the statistical fluctuation of the minimum count the reflection was treated as a non-observed one. The detailed explanation of the diffractometer-control program will be given elsewhere (Okaya, 1964*a*).

The structure was determined in a quite straightforward fashion by utilizing the phosphorus atom as a heavy atom. Its coordinates were studied first by a sharpened-peak origin-removed three-dimensional Patterson function. The phosphorus positions thus obtained were used in computing an approximate electron-density function from which the positions of other non-hydrogen atoms were easily deduced. These atomic coordinates, as well as the individual anisotropic temperature factors, were then refined with a full-matrix least-square program on an IBM 7094 computer (Okaya, 1963). After several cycles of iterative calculations, the positions of hydrogen atoms were determined by the usual F_o-F_c synthesis method. The coordinates of all the atoms, as well as their thermal parameters (only isotropic temperature factors used for hydrogen atoms), were again refined by the least-squares method. After several more cycles, no more progress in the refinement was noticed and the parameters at that stage were taken as the final values. The shifts in parameters in the final cycle are about 10^{-2} of their e.s.d.'s. The weighting scheme used was as follows: $\omega=1{\cdot}0$ for $F_{\text{obs}}<20{\cdot}0$, $\omega=20{\cdot}0/F_{\text{obs}}$ thereafter; unobserved reflections were given weights of zero. The atomic coordinates, the thermal parameters and their standard deviations are listed in Table 1. Comparison between the observed and calculated structure factors is given in Appendix I.* The conventional discrepancy index, $R=\Sigma||F_o|-|F_c||/\Sigma|F_o|$ is 0·046 including the non-observed reflections. The atomic scattering factors used in the computations were taken from the tables in *International Tables for X-ray Crystallography* (1959). No correction for anomalous dispersion was made to the phosphorus scattering factors. Interatomic distances and bond angles as shown in Figs. 1 and 2 were calculated with the use of the atomic coordinates listed in Table 1. Composite diagrams of the electron-density function are shown in Fig. 3.

Discussion

The molecule of 2-aminoethylphosphonic acid assumes a *trans* configuration around the central C(1)–C(2)

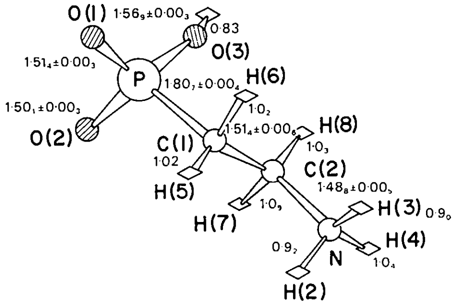

Fig. 1. Bond distances.

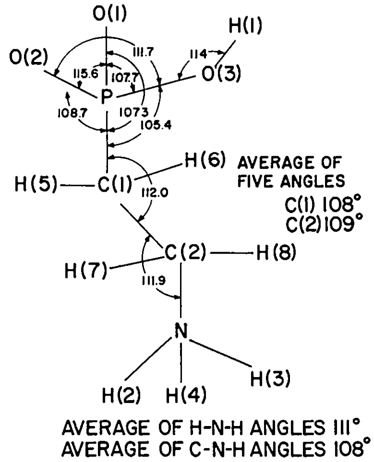

Fig. 2. Bond angles. Around C(1) and C(2) only the P–C(1)–C(2) and C(1)–C(2)–N angles are shown. The average of the remaining five angles around each atom is shown in the figure.

Table 1(*a*). *Atomic coordinates in fractions of cell edges, and their standard deviations in* 10^{-4} Å

Atoms	x	$\sigma(x)$	y	$\sigma(y)$	z	$\sigma(z)$
P	0·2261	5	0·0604	5	0·1638	6
O(1)	0·1549	17	−0·0079	18	0·2889	16
O(2)	0·1443	16	0·1413	18	0·0635	17
O(3)	0·3030	20	−0·0386	19	0·0689	19
C(1)	0·3475	22	0·1560	23	0·2539	24
C(2)	0·4337	23	0·2197	25	0·1386	23
N	0·5238	18	0·3091	20	0·2121	21
Hydrogen atoms on						
O(3)	0·313	382	−0·020	403	−0·022	450
N	0·480	508	0·377	543	0·248	441
	0·566	275	0·275	284	0·292	290
	0·584	404	0·332	373	0·121	433
C(1)	0·310	386	0·213	393	0·335	358
	0·397	332	0·093	308	0·318	297
C(2)	0·380	424	0·268	443	0·049	396
	0·482	271	0·152	275	0·078	264

Table 1(*b*). *Anisotropic temperature factors*

The β's are used in the usual expression; $\exp\{(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)\}$.

Atom	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
P	0·003171	0·003518	0·003248	−0·000323	−0·000794	0·000179
O(1)	0·005537	0·005851	0·004501	−0·003953	−0·000074	0·000870
O(2)	0·004335	0·005292	0·005307	0·001551	−0·001944	0·000457
O(3)	0·006474	0·004229	0·004421	0·001877	−0·000178	−0·000690
C(1)	0·003673	0·004458	0·004412	−0·001154	−0·001226	0·000620
C(2)	0·004625	0·005108	0·004193	−0·001563	−0·000312	0·000075
N	0·003437	0·004171	0·005201	−0·000492	0·000583	0·000005

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* Appendix I has been submitted with the manuscript and has been deposited as Document number 8553 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D.C. A copy may be secured by citing the Document number and by remitting $ 3·75 for photoprints, or $ 2·00 for 35-mm microfilm. Advance payment is required. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress.

bond; this is in contrast to the configuration of 2-aminoethanol phosphate in which the molecule takes a *cis* configuration around the methylene linkage (Kraut, 1963). A similar *cis* configuration is also found in choline *O*-sulfate, 2-trimethylaminoethanol sulfate (Okaya, 1964*b*). The positions of hydrogen atoms found in the structure substantiate the zwitterion configuration of the molecule. The P–O bond distances are divided into two categories: the shorter P–O(1) and P–O(2) bonds and the longer P–O(3)H bond. These P–O distances are quite similar to those found in the phosphate structure; the example shows a difference of about 0·06 Å between the P–O and P–OH bond lengths. The rest of the bond distances are also quite normal, so are the bond angles; the rather large O(1)–P–O(2) angle is the only exception. Table 2 is a detailed list of angles around C(1), C(2), and N.

Aside from the difference in the molecular configurations, the hydrogen bond system of the present crystal is almost a duplicate of that of the phosphate structure. As shown in Fig. 4, the nitrogen atom makes three hydrogen bonds of usual length, two of them to O(2), bonds *B* and *C* and one to O(1), bond *D*. A flattened tetrahedron is formed around the nitrogen atom by these three oxygen atoms and C(2). It is interesting to see that the angles between hydrogen bonds are greater than the usual tetrahedral angle and the angles involving C(2) are less than 100° (Fig. 5). The hydrogen atom on O(3) is involved in a hydrogen bond between O(3) and O(1), bond *A*, of $2{\cdot}53_4$ Å; this bond

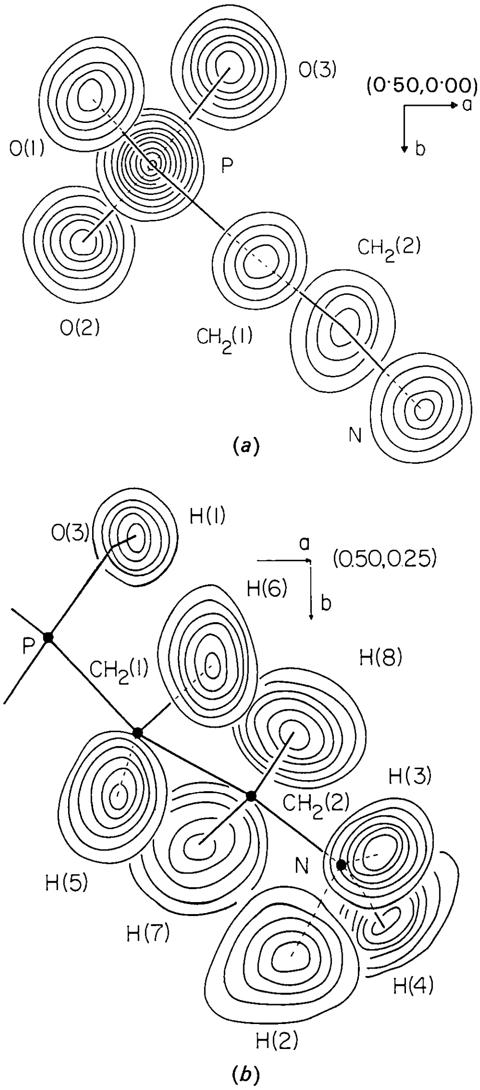

Fig. 3. (*a*) The three-dimensional electron density function showing the positions of heavy atoms. The contours are drawn at intervals of 2 e.Å^{-3} starting from 2 e.Å^{-3}. (*b*) Composite diagram of a three-dimensional $F_o - F_c$ synthesis showing hydrogen atoms. The contours are drawn at intervals of 0·1 e.Å^{-3} starting from 0·3 e.Å^{-3}.

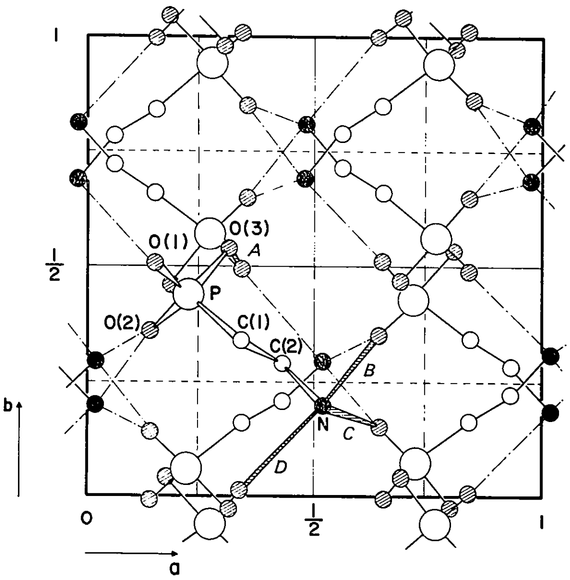

Fig. 4. Projection of the structure along the *c* axis. The N–H--O hydrogen bonds are shown by chain lines except those around the marked nitrogen atom, around which tapered lines are used to show the steric configuration. The O–H--O bond (*A*) is made by O(3) and is almost perpendicular to the plane of the projection. For N–H--O bonds, *B*, *C* and *D*, see text.

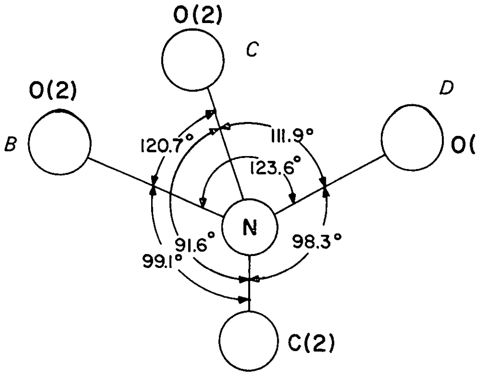

Fig. 5. Configuration of the three hydrogen bonds around the nitrogen atom.

Table 2. *Angles around* C(1), C(2), *and* N

Around C(1)		Around C(2)		Around N	
H–C(1)–H	102°	H–C(2)–H	102°	H–N–H	114°, 112°, 107°
H–C(1)–C(2)	115°, 112°	H–C(2)–N	112°, 109°	C(2)–N–H	112°, 111°, 101°
P–C(1)–H	112°, 103°	C(1)–C(2)–H	113°, 108°		
P–C(1)–C(2)	112·0°	C(1)–C(2)–N	111·9°		

is rather short and a similar hydrogen bond is found in the phosphate structure. The molecules are thus held together by a complicated network of N–H · · · O and O–H · · · O hydrogen bonds: the strong O–H · · · O hydrogen bonds around the twofold screw axis parallel to the *c* axis and the pyramidal arrangement of N–H · · · O bonds across the *a*-glide plane perpendicular to the *c* axis.

As in the case of the phosphate structure, planar configurations are found around the hydrogen bond acceptors, O(1) and O(2), with respect to the corresponding donors and the phosphorous atom. In Table 3 the least-squares planes and deviations of atoms from these planes are listed; other pertinent data are shown in Fig. 6. Around O(1), which is involved in two strong hydrogen bonds, all the angles are quite close to 120° and the four atoms make a perfect plane. In contrast to this condition, one of the hydrogen bonds around O(2) (Bond *B*, $2{\cdot}94_7$ Å) is rather weak, and the deviations from the planarity and from the ideal angle of 120° are noticeable. In the phosphate structure, no such weak hydrogen bond is formed and the planarity and the ideal angles are well kept. It may be interesting to study the hydrogen bond systems in similar crystals and establish the relationship between hydrogen bond strength and the related configuration around the hydrogen bond acceptor. As in the phosphate structure, the planes around O(1) and O(2) are almost perpendicular to each other with an angle of 94°. The hydrogen in the strong O–H · · · O bond sits almost in the line of O–O, making an angle of 174°. The N–H · · · O bonds are less linear and the angles around the hydrogen atoms are about 160° regardless of the hydrogen bond strength.

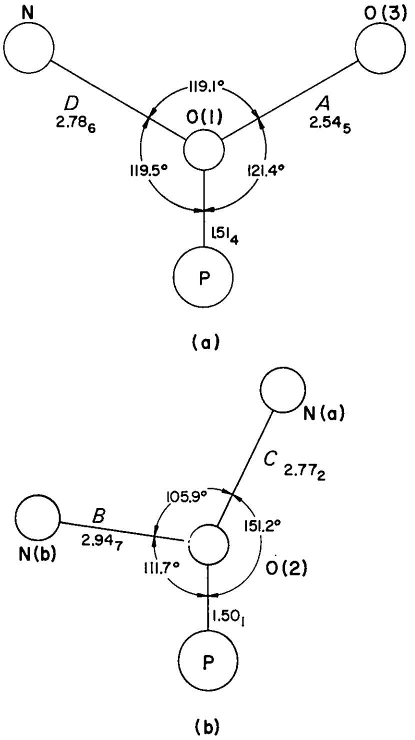

Fig. 6. The configurations of the hydrogen bond system around the two hydrogen-bond accepting oxygen atoms in the PO_3 group. (*a*) Around O(1). (*b*) Around O(2).

Table 3. *Deviations of atoms from the least-squares planes**

(*a*) Hydrogen bonds around O(1).

Plane: $0{\cdot}7238x-0{\cdot}6898y+0{\cdot}0159z=1{\cdot}2658$

Atom	P	O(1)	O(3)	N
Deviation	0·003	−0·006	0·002	0·002

(*b*) Hydrogen bonds around O(2).

Plane: $0{\cdot}7288x+0{\cdot}6585y-0{\cdot}1881z=1{\cdot}9087$

Atom	P	O(2)	N(a)†	N(b)†
Deviation	−0·045	0·078	−0·025	−0·011

* *x*, *y* and *z* are measured in Å.

† For these two nitrogen atoms refer to Fig. 6(*b*).

The author wishes to express his gratitude to Dr A. A. Benson of the Scripp Institution of Oceanography, La Jolla, California, U.S.A., and Dr M. Horiguchi of the Department of Agricultural Chemistry, University of Tokyo, Japan, for the samples of ciliatine used in this study and also for various interesting discussions on the biological aspect of the compound. The author is also grateful to N. R. Stemple for his help in collecting the intensity data on CCXD.

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