1974

Crystallographic Studies Of The Conformational And Phosphate Binding Properties Of Ethanolamines.

Richard Avery Hearn
University of Alabama at Birmingham

CRYSTALLOGRAPHIC STUDIES OF THE CONFORMATIONAL AND PHOSPHATE BINDING PROPERTIES OF ETHANOLAMINES

by

RICHARD AVERY HEARN

Submitted in partial fulfillment of the requirements
for the Doctor of Philosophy Degree in the
Department of Biochemistry, Graduate School
University of Alabama in Birmingham.

BIRMINGHAM, ALABAMA

1974

ACKNOWLEDGEMENTS

I sincerely thank Dr. Charles E. Bugg not only for his guidance in my graduate education but for his enthusiasm, optimism and dedication to his students.

The collaboration of Dr. Gerald R. Freeman and Miss Eileen Lynch in these crystallographic analysis is appreciated.

The patience, understanding and hard work of Mrs. Janet Saloom, Miss Mary Ann Comer and Miss Catherine Sims in the preparation of this dissertation was invaluable.

Finally this dissertation is dedicated to my daughter, Heather, who had very little to do with this manuscript but a great deal to do with its author. She makes it all worthwhile.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

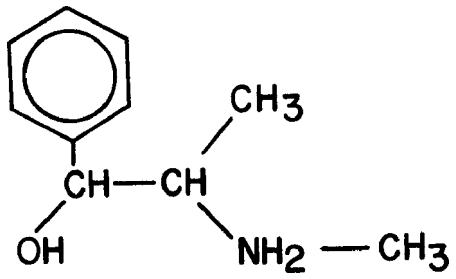
The β-ethanolamine moiety is a functional group which occurs in several biological molecules that have been implicated in phosphate binding processes (Figure 1). Particularly notable examples are the adrenergic phenylethanolamines (e.g. epinephrine and norepinephrine) and the amino acid hydroxylysine, a component of collagen.

The adrenergic phenylethanolamines are thought to be stored as complexes with adenosine triphosphate (ATP) (2, 13) and it has been postulated that similar ATP-phenylethanolamine complexes are involved at the adrenergic receptor sites (35). Both IR (21, 22) and NMR (38) studies show that phenylethanolamines bind only to the phosphate anion moieties of ATP and AMP in aqueous solution. These studies also show that compounds which are identical to the phenylethanolamines except for the absence of α-hydroxyl group, do not bind to ATP in aqueous systems. This indicates that the intact ethanolamine moiety is necessary for phosphate-binding.

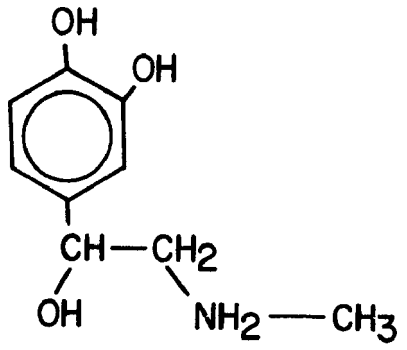
The ethanolamine sidechain of collagen has been implicated in interactions between collagen and the phosphate salts of bones and teeth (28, 29, 32). Bone consists of calcium phosphate minerals deposited in

Figure 1.

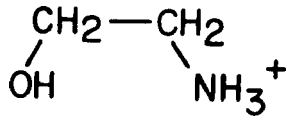
The structural formula of the three ethanolamines relevant to this study. Phenylethanolamines refer to ethanolamines in which a phenyl group replaces a hydrogen group on the carbon that bears the hydroxyl substituent (i.e. ephedrine and epinephrine).



Ephedrine



Epinephrine



Ethanolamine

organic matrices of which collagen is the major component. Various physical and chemical studies have suggested that mineral deposition in bone may be partially controlled by the collagen matrix (28, 29, 42). Electron microscopic studies indicate that, in mature bone, crystallites of mineral are not only in contact with collagen but are oriented with respect to the collagen fibrils (29); therefore, it is possible that the physical and mechanical properties of mature bone are influenced by mineral-collagen interactions. It has been demonstrated that collagen binds phosphate ions (9, 10), and this binding has been linked to the availability of lysine and hydroxylysine residues. This evidence suggests that the ethanolamine sidechain of hydroxylysine residues in collagen may be involved in phosphate interactions. Our work with space-filling, molecular models suggest that the ethanolamine moieties of hydroxylysine residues might provide an especially effective site for hydrogen bonding to phosphate anions.

Despite the evidence that phosphate-ethanolamine interactions are of considerable biological importance, little is known about the specific factors that govern these interactions. To investigate the physical and structural factors controlling phosphate interactions with ethanolamines, I have undertaken a series of crystallographic studies of ethanolamine-phosphate salts. By using

x-ray diffraction methods to examine the detailed crystal structures of these salts, I have been able to study the phosphate-ethanolamine interactions with high precision at the molecular level. X-ray crystallography not only allows one to view the conformational and structural features of biological molecules, but this approach also can be used to investigate the details of intermolecular associations. The study of a biological complex by x-ray crystallography can furnish the following information: 1) it shows that the interaction can occur under the constraints of a solid-state environment, 2) it elucidates relationships between the conformations of the moieties involved and their ability to form these biological complexes, and 3) it details the types of interactions that stabilize the complex. Therefore, it seems that this approach, particularly when combined with spectroscopic results from aqueous solution studies (21, 22, 38), offers a feasible method for answering relevant questions regarding the details of phosphate-ethanolamine interactions.

As part of this study of the factors controlling phosphate binding to ethanolamine, I determined the crystal structures of ephedrine dihydrogen phosphate, ephedrine monohydrogen phosphate dihydrate and ethanolamine monohydrogen phosphate. These crystallographic studies permitted the examination of phosphate interactions with

ethanolamine moieties in a variety of different solid-state environments. These results indicate that ethanolamines are particularly well suited for hydrogen-bonding to phosphate ions. In all three crystal structures, I find that the ethanolamine moieties assume a characteristic conformation that leads to simultaneous hydrogen-bonding from the amino and from the hydroxyl groups to oxygen atoms of the phosphate ions. Examination of the conformations assumed by ethanolamines in a variety of other crystal structures, that are without phosphate interactions, suggests that ethanolamines generally assume a rigid conformation that would lead to strong and specific interactions with phosphate ions. In this thesis, I discuss the results of my crystallographic analyses, describe the general conformational and phosphate-binding properties of ethanolamine, and speculate about the implications of these properties as factors in controlling the biological roles of adrenergic phenylethanolamines and hydroxylysine residues of bone collagen.

CRYSTALLOGRAPHIC STUDIES OF ETHANOLAMINE PHOSPHATE SALTS.

Spectroscopic studies indicated that the binding of adrenergic phenylethanolamines to ATP is mediated through specific interactions between the ethanolamine moieties of the phenylethanolamines and a single PO_4 group of ATP (21, 22, 38). Therefore, any phenylethanolamine that could be crystallized as a phosphate salt should serve as a suitable model system for examining the general factors controlling the binding of phenylethanolamines to ATP. I selected ephedrine as my model system for several reasons:

1. I was able to obtain good crystals of both the monohydrogen phosphate and dihydrogen phosphate salts of ephedrine. Consequently, by utilizing this system, I was able to examine the effects that different degrees of phosphate protonation have on the interactions.
2. Detailed molecular orbital calculations of the conformational properties of ephedrine have been published (16) and allow a final comparison between the crystallographic and theoretical results.
3. The crystal structure of ephedrine as the hydrochloride salt (3) has been reported; thus it

was possible for me to recognize immediately any specific structural changes that were attributable to phosphate interactions.

4. Crystallographic data for a number of closely related phenylethanolamines were available and could be used, along with my structural results for ephedrine, to develop a general relationship between phenylethanolamine conformational properties and the ability of these molecules to interact with phosphate ions.

As shown in Figure 1, ephedrine is an ethanolamine structurally related to hydroxylysine (R group has ethanolamine moiety). Assuming that only the ethanolamino portion of phenylethanolamine participates in phosphate interactions, then any data obtained about phosphate binding to ephedrine should also be applicable to hydroxylysine. Therefore, it was reasonable to assume that the ephedrine phosphate crystal structures would probably shed light on the factors that might influence interactions of phosphate minerals with the hydroxylysine residues of bone collagen. Unfortunately, one serious objection could be raised to this assumption. Early in my crystallographic studies of ephedrine it became obvious that phenylethanolamines assume a characteristic conformation, and it was soon apparent that this particular conformation was required for strong interactions of phosphate ions with the ethanolamine moieties. How could I be certain that this

conformation was not merely a consequence of steric interactions resulting from bulky phenyl groups in the molecules? This was a critical question, since it implied that the sidechains of hydroxylysine might prefer a conformation that is completely different from that preferred by ephedrine and other phenylethanolamines. If this were so, then the phosphate-ephedrine interactions might bear little resemblance to phosphate-hydroxylysine interactions.

To investigate the possible effects that the phenyl groups of phenylethanolamines exert on the conformational properties of the molecules, I also determined the crystal structure of the phosphate salt of β-ethanolamine. As shown in Figure 1, this simple ethanolamine contains no bulky groups to influence the conformational properties of the molecule, so interactions observed between phosphate ions and β-ethanolamine should be of immediate relevance to hydroxylysine. Therefore, I felt that crystallographic studies of ephedrine dihydrogen phosphate, ephedrine monohydrogen phosphate, and β-ethanolamine monohydrogen phosphate should be sufficient to permit me to identify those structural features that are of general importance in controlling interactions between phosphates and various ethanolamines.

I. Crystal Structure of Ephedrine Dihydrogen Phosphate

A. Experimental

Ephedrine dihydrogen phosphate, isolated by lyophilizing an aqueous solution containing equimolar quantities of ephedrine and phosphoric acid, was crystallized by slowly cooling a hot, saturated, aqueous ethanol solution. Two types of crystals were obtained: monoclinic needles and orthorhombic plates. Density measurements and elemental analyses showed that the ephedrine:phosphoric acid molar ratio is 1:1 for the needles and 2:1 for the plates. The needles were used for the structure analysis reported here.

Weissenberg and oscillation photographs showed the crystals to be monoclinic. Space groups C2, Cm, and C2/m are indicated by the systematic absence of reflections $hk\ell$ with $h + k = 2n + 1$; since ephedrine is optically active, the space group is C2. A section with the approximate dimensions 0.3, 0.2, and 0.1 mm was cut from one of the larger needles and then mounted on a Picker FACS-1 diffractometer with its b axis (the needle axis) slightly inclined to the ϕ axis of the diffractometer. Approximate cell parameters for use in collection of intensity data were calculated by a least-squares analysis

of the angular setting for eight low-angle $CuK\alpha_1$ (λ = 1.5418 Å) reflections.

Intensity data were collected with the diffractometer, by use of a scintillation counter, nickel-filtered copper radiation, and a θ-2θ scanning technique. Measurements were made for the 1180 reflections with $2\theta < 128°$. Intensity values were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus an additional term $(0.03S)^2$, being the scan counts. The intensities and their variances were corrected for Lorentz and polarization factors, and absorption corrections were applied by use of the program ORABS (37). Structure factors and variances were placed on an approximately absolute scale by means of a Wilson plot (39).

Immediately after data collection, accurate values for the cell parameters were obtained by a least-squares analysis of 2θ values for fifteen high-angle reflections (λ = 1.54051 Å) measured with the diffractometer. Crystal data are listed in Table 1.

A suitable trial structure was found by the heavy atom method; the x and z coordinates for the phosphorous atoms were obtained from a sharpened, three-dimensional Patterson map; the phosphate oxygen atoms were located in a Fourier map calculated by use of phase angles derived from the phosphorous atom; and the remaining nonhydrogen atoms were located in a Fourier map calculated with phase

angles derived from the phosphate anion. The structure was refined by use of a modified version of the full matrix least-squares program ORFLS (4). The quantity minimized was $\Sigma w(Fo^2-(1/k)Fc^2)^2$, where k is a scale factor and the weight w is equal to $(1/\sigma(Fo^2))^2$. Atomic scattering factors for the nonhydrogen atoms, as well as the real and imaginary anomalous dispersion corrections for phosphorous and oxygen, were from the International Tables for X-Ray Crystallography (1962) (42). Hydrogen scattering factors were from Stewart, Davidson, and Simpson (1965) (33). Hydrogen atoms were located in a difference Fourier map calculated during the final stages of refinement.

I refined all positional parameters, anisotropic temperature parameters for the nonhydrogen atoms, isotropic temperature factors for the hydrogen atoms, and Zachariasen's extinction parameter g (41) (as formulated by Coppens and Hamilton, 1970 b). Heavy and hydrogen atoms were refined in alternate cycles. The final R index $(\Sigma||Fo|-|Fc||/\Sigma|Fo|)$ for all reflections is 0.026; the goodness-of-fit, $(\Sigma(1/\sigma^2(Fo^2))(Fo^2-Fc^2/k^2)^2/(m-s))^{1/2}$, is 1.81. During the final cycle of refinement no parameter shifted more than one-fifth of its standard deviation. A final difference Fourier map showed no peaks or troughs exceeding 0.2 e/Å in magnitude.

At the conclusion of refinement, the coordinates were inverted and the structure of the enantiomorph

((+)-ephedrine) was refined. The enantiomorph refined to R = 0.027, and a goodness-of-fit of 1.86.

B. Results

The nonhydrogen atom parameters and their standard deviations are listed in Table 2; the average, estimated standard deviations in these positional parameters range from 0.0015 to 0.004 Å. The hydrogen-atom parameters and their standard deviations are given in Table 3; the estimated errors in the hydrogen-atom positional parameters range from 0.03 to 0.06 Å. Table 4 lists observed and calculated structure factors.

Figure 2 shows the conformation of the ephedrine molecule, including the ellipsoids of thermal vibration (14). Figure 3 shows the conformation and thermal ellipsoids for the phosphate ions. Bond lengths are given in Table 5 and bond angles in Table 6. The conformation and the bond lengths and angles of ephedrine are in agreement with those in the crystal structure of ephedrine hydrochloride (3) except for a difference of 47° in the torsion angle about the C(7)-O(11) bond. As expected, the phenyl ring is planar within experimental error and has no heavy-atom deviations exceeding 0.007 Å. Atom C7 is displaced 0.05 Å from the phenyl plane.

Figure 4 depicts the crystal packing. The hydrophobic phenyl and methyl groups are clustered in the central region of the unit cell, and the polar amino,

hydroxyl, and phosphate moieties are arranged in layers parallel to the ab plane. Thus the structure consists of alternate hydrophobic and polar layers; the hydrophobic portions are sandwiched between polar regions. Figure 4 also shows the hydrogen-bonding scheme. Hydrogen-bond lengths and angles are given in Table 7. All hydrogen atoms covalently bonded to oxygen or nitrogen atoms participate in hydrogen bonding. The nitrogen and oxygen atoms of the ethanolamine moiety donate hydrogen bonds to phosphate oxygen atoms and the phosphate hydrogen atoms are utilized in phosphate-phosphate hydrogen bonding. Phosphate anions are hydrogen bonded around two-fold rotation axes to form dimers that involve two short (2.55 Å) hydrogen bonds (Figure 4). The second phosphate hydrogen atom is involved in hydrogen bonding between phosphate dimers. The phosphate-phosphate hydrogen bonding results in cohesive, continuous sheets of phosphate ions running parallel to the ab plane. Figure 5 depicts hydrogen bonding within these sheets.

Figure 2.

The conformation of the ephedrine cation. Heavy atoms are represented by ellipsoids defined by the principal axes of thermal vibration and scaled to include 50% probability. Hydrogen atoms are represented by spheres of 0.1 Å radius.

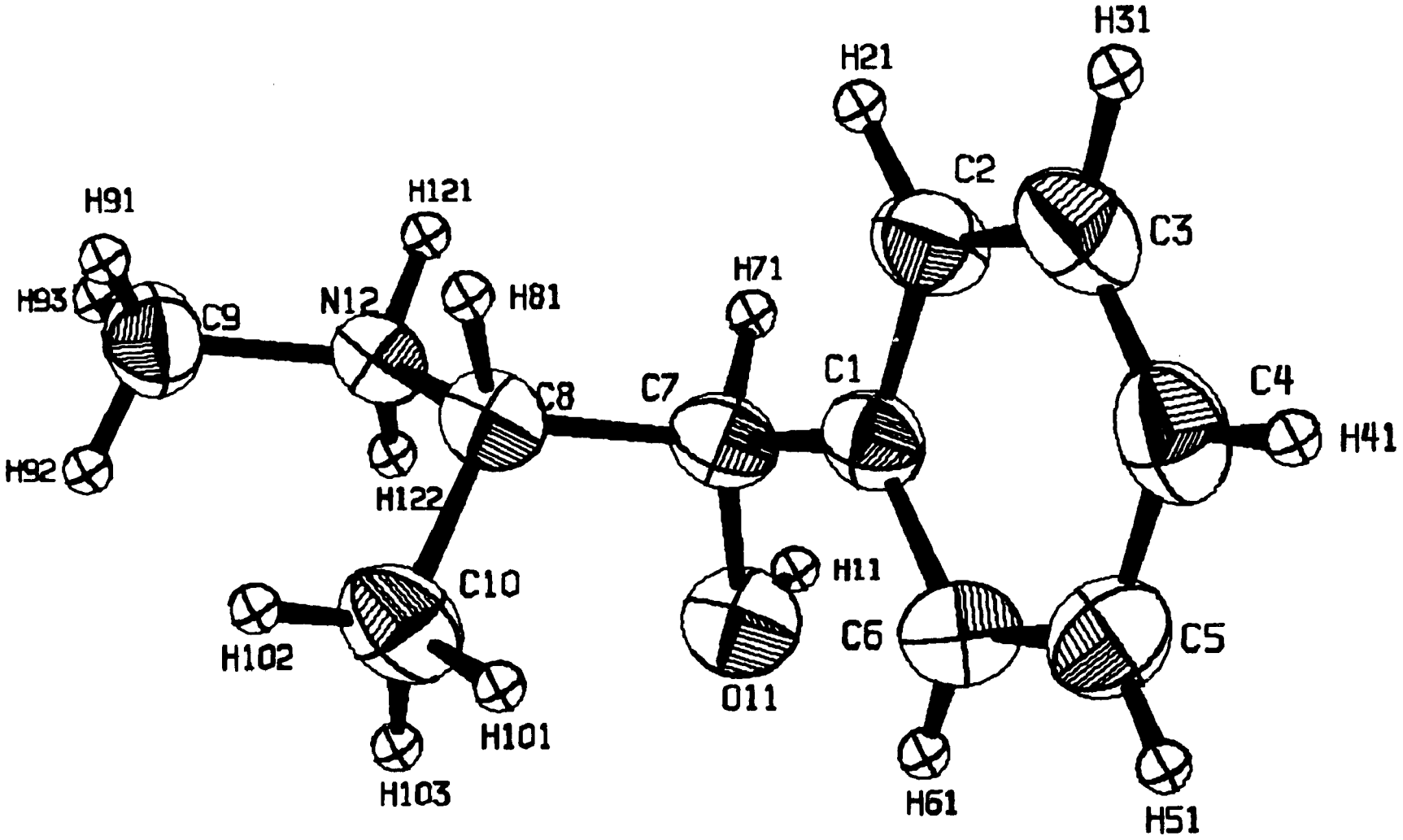

H31
H21
C2
C3
H91
H121
H71
H93
C9
N12
H81
C1
C4
C7
C8
H41
H92
H122
H11
C10
C6
C5
H102
O11
H101
H61
H103
H51

Figure 3.

Phosphate anions hydrogen-bonded across a two-fold rotation axis. OP3-OP1 distances are 2.55 Å. The phosphorous and oxygen atoms are represented by thermal ellipsoids scaled to include 50% probability.

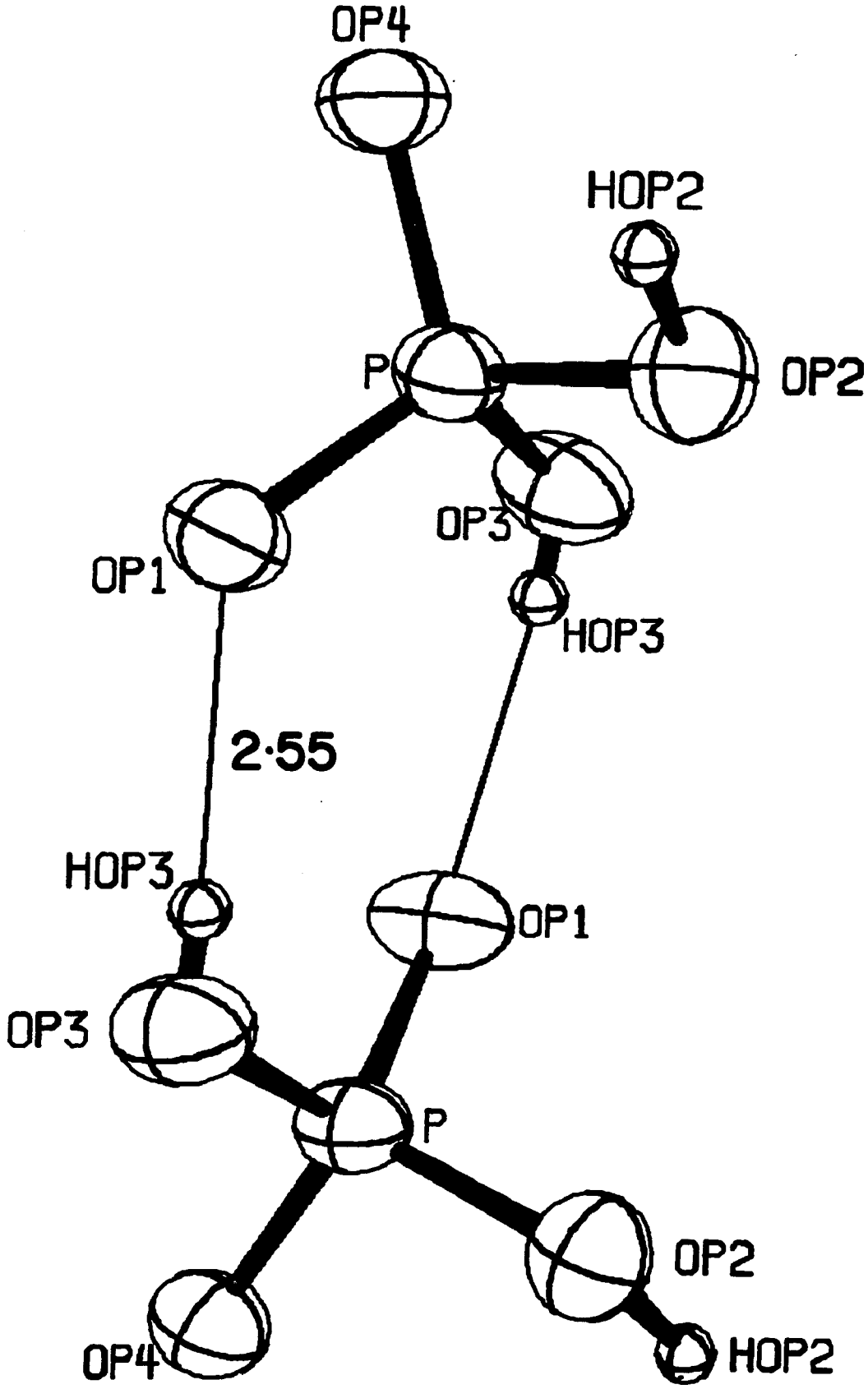

OP4
HOP2
P
OP2
OP3
OP1
HOP3
2·55
HOP3
OP1
OP3
P
OP2
OP4
HOP2

Figure 4.

The crystal structure of ephedrine dihydrogen phosphate viewed down the b axis. Thin lines represent hydrogen bonds; donor-acceptor distances are shown.

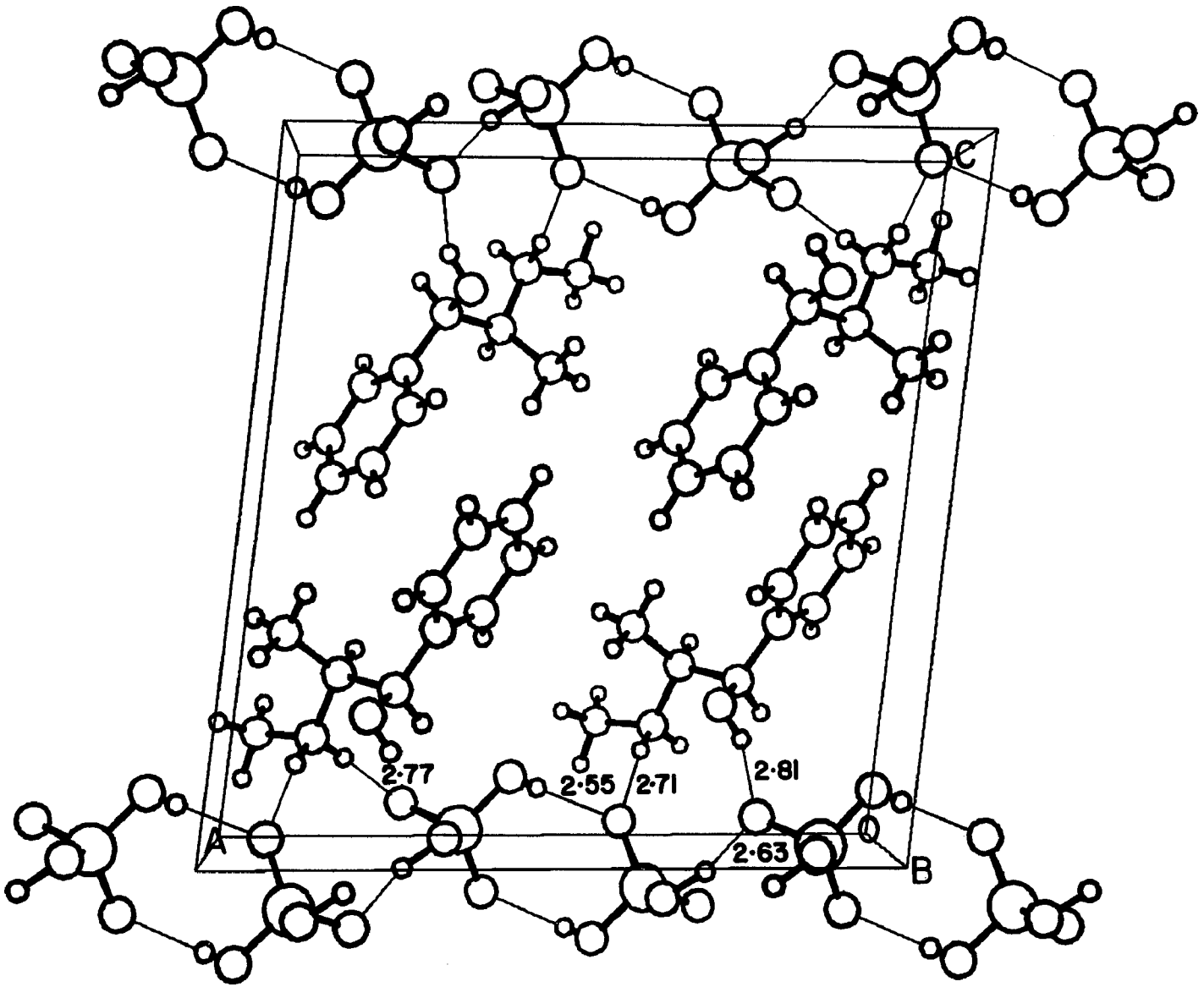

C
A
O
B
2·77
2·55
2·71
2·81
2·63

Figure 5.

Sheets of hydrogen-bonded phosphate anions, as viewed down the c axis. The sheets are parallel to the ab plane. The thin lines represent bonds.

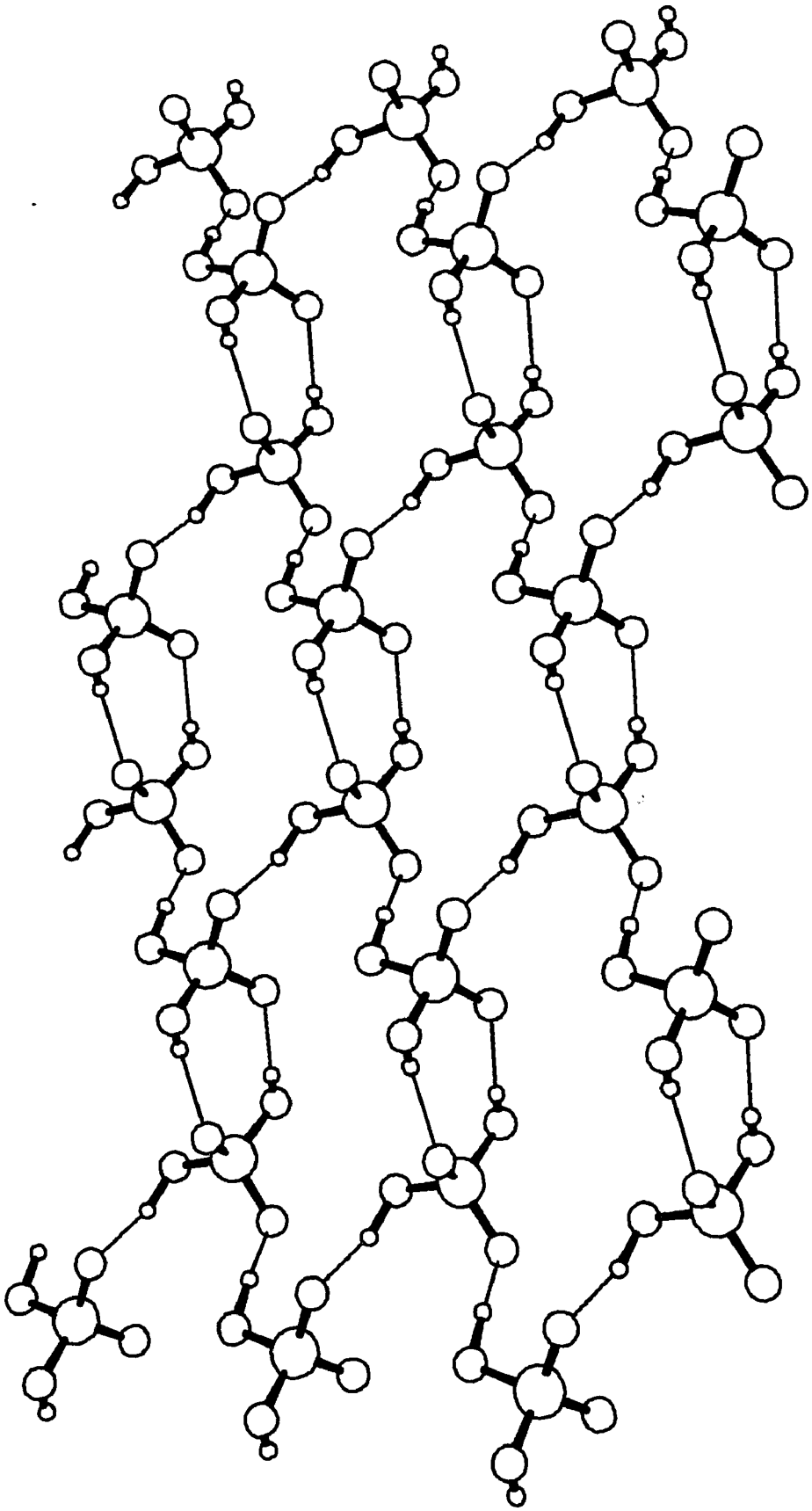


TABLE 1. Crystal data for ephedrine dihydrogen phosphate.

Stoichiometry	$C_{10}H_{16}NO \cdot H_2PO_4$
Z	4
Space Group	C2
a	14.738(4) Å
b	5.710(6)
c	15.302(4)
β	97.17(2)°
ρ (calculated)	1.368 g cm^{-3}
ρ (observed)	1.37
μ	20.4 cm^{-1}

(The unit-cell parameters were measured at 25 ± 3°C. The reported standard deviations are five times those obtained from the least-squares analysis. The density was measured by flotation in a mixture of benzene and ethylene dibromide).

TABLE 2. The final heavy atom parameters and their standard deviations. All values have been multiplied by 10^4. The temperature factors are in the form $T=\exp(-\beta_{11}h^2-\beta_{22}k^2-\beta_{33}\ell^2-2\beta_{12}hk-2\beta_{13}h\ell-2\beta_{23}k\ell)$.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
P	1391(1)	2093	405(1)	24(1)	253(2)	28(1)	-3(1)	3(1)	6(1)
OP1	942(1)	1359(4)	-485(1)	28(1)	409(8)	30(1)	-1(2)	3(1)	-27(2)
OP2	1590(1)	4809(4)	409(1)	44(1)	251(7)	41(1)	3(2)	5(1)	0(2)
OP3	734(1)	1848(5)	1120(1)	32(1)	443(8)	31(1)	-21(3)	5(1)	-5(2)
OP4	2267(1)	837(4)	726(1)	30(1)	277(6)	41(1)	9(2)	0(1)	5(2)
C6	2177(2)	7795(5)	3797(2)	52(1)	272(10)	42(1)	-14(3)	5(1)	-4(3)
C5	1742(2)	7913(6)	4549(2)	57(2)	356(12)	44(1)	0(3)	8(1)	-33(3)
C4	1162(2)	6155(7)	4738(2)	41(1)	482(13)	41(1)	73(4)	8(1)	-10(4)
C3	1017(2)	4259(7)	4181(2)	39(1)	438(13)	55(2)	-26(4)	12(1)	2(4)
C2	1450(2)	4138(6)	3430(2)	42(1)	331(10)	46(1)	25(3)	5(1)	-16(3)
C1	204(2)	5884(5)	3234(2)	35(1)	271(8)	33(1)	-2(3)	-2(1)	1(3)
C7	2534(2)	5668(5)	2429(2)	40(1)	289(10)	30(1)	-15(3)	0(1)	1(3)
O11	2852(1)	7872(4)	2150(1)	57(1)	309(8)	39(1)	-8(2)	3(1)	33(2)
C8	3367(2)	4032(5)	2604(2)	40(1)	269(9)	30(1)	-9(3)	2(1)	16(2)
N12	3643(1)	3316(5)	1727(1)	31(1)	264(7)	33(1)	-11(2)	3(1)	4(2)
C9	4464(2)	1813(7)	1744(2)	41(1)	334(11)	54(1)	15(4)	5(1)	18(4)
C10	4165(2)	5147(7)	3177(2)	46(1)	451(13)	37(1)	-11(4)	-8(1)	-8(3)

TABLE 3.

The final hydrogen atom parameters and their estimated standard deviations. The positional parameters have been multiplied by 10^3.

Atom	x	y	z	β
HOP2	213(2)	509(8)	003(2)	7.3(1.0)
HOP3	032(2)	181(9)	097(2)	7.5(.9)
H11	249(3)	803(10)	169(3)	9.3(1.2)
H21	138(2)	269(6)	307(2)	4.9(.7)
H31	062(2)	292(6)	432(2)	4.4(.6)
H41	086(2)	632(8)	529(2)	5.9(.7)
H51	184(2)	909(7)	488(2)	6.1(.8)
H61	255(2)	890(6)	367(2)	5.1(.7)
H71	210(2)	486(5)	196(2)	3.3(.5)
H81	320(2)	250(5)	290(2)	3.8(.6)
H91	445(2)	056(7)	211(2)	6.7(.9)
H92	502(2)	266(8)	192(2)	7.7(1.0)
H93	460(2)	139(7)	110(2)	6.2(.8)
H101	394(2)	589(7)	371(2)	5.5(.7)
H102	465(2)	407(6)	336(2)	5.4(.7)
H103	446(2)	658(9)	289(2)	7.4(.9)
H121	316(2)	250(6)	142(2)	4.6(.6)
H122	381(2)	459(6)	141(1)	5.2(.7)

TABLE 4.

Observed and calculated structure factors. From left to right, the columns contain values of h, 10Fo, 10Fc.

[illegible]

TABLE 5.

Bond distances. Standard deviations in bond lengths that involve only nonhydrogen atoms are about 0.005 Å. Standard deviations in bond lengths that involve hydrogen atoms are about 0.06 Å.

BOND	DISTANCE	BOND	DISTANCE
P - OP1	1.498	C7 - H71	1.01
P - OP2	1.578	C8 - H81	1.03
P - OP3	1.555	N12 - H121	.93
P - OP4	1.505	N12 - H122	.93
C5 - C6	1.387	C6 - H61	.87
C6 - C1	1.389	C5 - H51	.85
C5 - C4	1.373	C4 - H41	1.01
C3 - C4	1.379	C3 - H31	1.00
C2 - C3	1.384	C2 - H21	.99
C1 - C2	1.382	O11 - H11	.85
C1 - C7	1.512	C10 - H101	1.01
C7 - O11	1.427	C10 - H102	.95
C7 - C8	1.539	C10 - H103	1.05
C8 - C10	1.506	C9 - H91	.91
C9 - N12	1.481	C9 - H92	.96
C8 - N12	1.506	C9 - H93	1.07
		OP2 - HOP2	1.06
		OP3 - HOP3	.63

TABLE 6.

Bond angles. Standard deviations in bond angles that involve only nonhydrogen atoms are about 0.4°. Standard deviations in bond angles that involve hydrogen atoms are about 4°.

ATOMS	ANGLE	ATOMS	ANGLE
OP1 - P - C9	109.8	HOP2 - OP2 - P	107.6
OP1 - P - OP3	111.8	HOP3 - OP3 - P	113.6
OP1 - P - OP4	115.3	H21 - C2 - C1	120.6
OP2 - P - OP3	102.6	H21 - C2 - C3	118.2
OP2 - P - OP4	108.4	H31 - C3 - C4	120.9
OP3 - P - OP4	108.2	H31 - C3 - C2	119.1
C6 - C1 - C2	118.6	H41 - C4 - C5	117.4
C6 - C1 - C7	121.5	H41 - C4 - C3	122.8
C2 - C1 - C7	119.9	H51 - C5 - C6	118.5
C3 - C2 - C1	120.9	H51 - C5 - C4	121.2
C4 - C3 - C2	120.0	H61 - C6 - C5	121.2
C5 - C4 - C3	119.8	H61 - C6 - C1	118.4
C6 - C5 - C4	120.3	H71 - C7 - C1	113.1
C5 - C6 - C1	120.5	H71 - C7 - O11	105.2
C1 - C7 - O11	112.4	H71 - C7 - C8	112.4
C1 - C7 - C8	111.2	H81 - C8 - C7	111.2
O11 - C7 - C8	107.6	H81 - C8 - N12	105.5
C7 - C8 - N12	108.0	H81 - C8 - C10	108.4
C7 - C8 - C10	112.9	H91 - C9 - H92	108.4
N12 - C8 - C10	110.6	H91 - C9 - H93	114.8
C8 - N12 - C9	117.0	H91 - C9 - N12	112.3
		H92 - C9 - H93	96.7
		H92 - C9 - N12	111.6
		H92 - C9 - N12	111.6
		H101 - C10 - H102	109.6
		H101 - C10 - H103	101.7
		H101 - C10 - C8	109.5
		H102 - C10 - H103	107.4
		H102 - C10 - C8	113.4
		H103 - C10 - C8	114.6
		H11 - O11 - C7	98.6
		H121 - N12 - H122	111.0
		H121 - N12 - C8	108.8
		H121 - N12 - C9	106.5
		H122 - N12 - C8	112.0
		H122 - N12 - C9	101.2

TABLE 7. Hydrogen bond distances and angles.

DONOR ATOM	HYDROGEN ATOM	ACCEPTOR ATOM	DISTANCES (A) DONOR-ACCEPTOR	DISTANCES (A) HYDROGEN-ACCEPTOR	DONOR-HYDROGEN-ACCEPTOR
OP3	HOP3	OP1 (a)	2.554	1.93	175
OP2	HOP2	OP4 (b)	2.632	1.60	164
N12	H122	OP1 (b)	2.702	1.81	160
N12	H121	OP4 (c)	2.772	1.84	176
O11	H11	OP4 (d)	2.808	2.17	133

(a) -x, y, -z

(b) 1/2 - x. 1/2 + y, -z

(c) x, y, z

(d) x, y + 1, z

II. Ephedrine Monohydrogen Phosphate Dihydrate

A. Experimental

Ephedrine phosphate was obtained by lyophilizing an aqueous solution that contained equimolar quantities of ephedrine and phosphoric acid. Slow cooling a hot aqueous ethanol solution of ephedrine phosphate produced two types of crystals: clear needles of ephedrine dihydrogen phosphate and clear plates of ephedrine monohydrogen phosphate monohydrate. Weissenberg and oscillation photographs showed the plates to be orthorhombic; the space group is $P2_12_12_1$ as indicated by the systematic absence of reflections h00 with h odd, 0k0 with k odd, and 00ℓ with ℓ odd. A crystal fragment with dimensions of 0.19, 0.17, and 0.08 mm was mounted on a Picker FACS-1 diffractometer with its b axis slightly inclined to the ϕ axis of the diffractometer. Unit-cell parameters were determined by a least-squares analysis of the angular settings for six high-angle ($CuK\alpha_1$, λ = 1.54051 Å) reflections measured with the diffractometer. Crystal data are listed in Table 8.

Intensity data were collected with the diffractometer by use of a scintillation counter, nickel-filtered copper radiation, and a θ-2θ scanning technique. Measurements were made for the 2403 unique reflections with

$2\theta<128°$. Those reflections with scan counts less than background levels were assigned intensity values of 0.0 and were retained in all subsequent calculations. The intensity values were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus an additional term $(0.03S)^2$, S being the scan counts. The intensities and variances were corrected for Lorentz and polarization factors, absorption corrections were applied by using the computer program ORABS (37), and structure factors and their variances were scaled by means of a Wilson plot.

Trial coordinates for the phosphorous atom and for the four oxygen atoms of the phosphate group were obtained by direct methods, with the use of the computer program MULTAN (19). The other nonhydrogen atoms were located in a Fourier map that was calculated with phase angles derived from the five heavy atoms of the phosphate group. The trial structure was refined by use of a modified version of the full-matrix least-squares program ORFLS (4). The quantity minimized was $\Sigma \underline{w}(Fo^2-(1/k)Fc^2)^2$, where k is a scale factor and weight $\underline{w}$ is equal to $(1/\sigma(Fo^2))^2$. All measured reflections regardless of their $1/\sigma(I)$ ratio, were included in the refinement. Atomic scattering factors for the nonhydrogen atoms were taken from International Tables for X-Ray Crystallography (1962); anomalous dispersion corrections for these atoms were those of Cromer

and Liberman (7). The hydrogen-atom scattering factors were taken from Stewart, Davidson, and Simpson (33). Hydrogen atoms were located in a difference Fourier map that was calculated during the final stages of refinement. The last cycles of refinement included all positional parameters, anisotropic temperature parameters for the non-hydrogen atoms, isotropic temperature factors for the hydrogen atoms, and Zachariasen's extinction parameter g (41) (as formulated by Coppens and Hamilton (6)). Because of the limited core-storage capacity of the computer it was impracticable to refine all parameters simultaneously; consequently, the parameters were divided into three blocks, those for each of the ephedrine cations in separate blocks, and those for the phosphate ion and the water molecule in a third block. The blocks of parameters were refined in successive cycles. The final R index ($\Sigma||Fo|-|Fc||/\Sigma|Fo|$) for all reflections is 0.033; the goodness-of-fit $(\Sigma(1/\sigma^2 Fo^2))(Fo^2-Fc^2/k^2)/(m-s))^{1/2}$, where m is the number of reflections used and s is the number of parameters refined, is 1.38. During the last cycle of refinement, no atomic parameter shifted more than one-fourth of its standard deviation. A final difference Fourier map showed no peaks or troughs exceeding .2 e/$\mathring{A}^3$ in magnitude.

B. Results

Table 9 lists the final heavy-atom parameters and their estimated standard deviations. Table 10 gives the

hydrogen-atom parameters and their estimated standard deviations. The estimated errors in positional coordinates are less than 0.005 Å for all the hydrogen atoms. Table 11 lists observed and calculated structure factors.

Figure 6 shows the conformations and the thermal ellipsoids of the two crystallographically-independent ephedrine cations (designated ephedrine-A and ephedrine-B). Conformational torsion angles are shown in Figure 9, bond lengths are given in Table 12, and bond angles are listed in Table 13. The phenyl groups are planar within experimental error: no atoms deviate from the phenyl planes by more than 0.01 Å.

The crystal-packing and hydrogen-bonding schemes are shown in Figure 7. The phenyl- and the methyl-hydrophobic groups are clustered together; the water molecules, the phosphate anions, and the hydroxyl and amino groups are hydrogen bonded together. The resultant packing scheme consists of alternate layers of polar and hydrophobic groups, with the layers running parallel to the ab plane. Table 14 gives hydrogen-bond distances and angles. As shown in Figure 8, each of the ephedrine cations forms two hydrogen bonds one from both the amino and hydroxyl groups to the phosphate anion.

TABLE 8. Crystal data for ephedrine monohydrogen phosphate dihydrate.

Stoichiometry	$(C_{10}H_{16}NO^{+})_2\ HPO_4^{2-}(H_2O)_2$
Z	4
Space Group	$P2_12_12_1$
a	7.094(1)
b	11.290(1)
c	29.567(5)
ρ (calculated)	1.253 g cm^{-3}
ρ (observed)	1.26 g cm^{-3}
μ	13.8 cm^{-1}

(Reported standard deviations in unit-cell parameters are three times those obtained from the least-squares analysis. Density was measured by flotation in a mixture of carbon tetrachloride and benzene.)

TABLE 9.

Final heavy-atom parameters and their estimated standard deviations. All values have been multiplied by 10^4. Temperature factors are in the form $T = \exp(-\beta_{11}h^2-\beta_{22}k^2-\beta_{33}\ell^2-2\beta_{12}hk-2\beta_{13}h\ell-2\beta_{23}k\ell)$. Final value of the isotropic extinction parameter (g) is 0.278.

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
				Ephedrine A					
C(1)	4830 (3)	2243 (2)	5985 (1)	113 (5)	71 (2)	7 (1)	7 (3)	2 (1)	2 (1)
C(2)	5423 (5)	1306 (3)	5717 (1)	202 (7)	88 (3)	9 (1)	22 (4)	7 (1)	1 (1)
C(3)	6321 (5)	1509 (4)	5307 (1)	251 (9)	131 (4)	10 (1)	16 (5)	14 (2)	3 (1)
C(4)	6629 (5)	2647 (4)	5165 (1)	197 (7)	168 (5)	8 (1)	−24 (5)	9 (1)	4 (1)
C(5)	6062 (6)	3579 (4)	5425 (1)	261 (9)	115 (4)	11 (1)	−67 (5)	−1 (2)	10 (1)
C(6)	5169 (5)	3388 (3)	5838 (1)	241 (8)	82 (3)	9 (1)	−32 (4)	3 (1)	39 (1)
C(7)	3827 (4)	1982 (2)	6425 (1)	140 (6)	47 (2)	7 (1)	−1 (3)	−3 (1)	1 (1)
C(8)	1667 (4)	1951 (2)	6360 (1)	134 (5)	64 (2)	6 (1)	−4 (3)	−2 (1)	−1 (1)
O(1)	4303 (3)	2823 (2)	6762 (1)	185 (4)	61 (2)	7 (1)	−29 (2)	−4 (1)	−1 (1)
C(9)	−1239 (4)	1367 (3)	6791 (1)	134 (6)	98 (3)	14 (1)	−42 (4)	−1 (1)	2 (1)
N(1)	0811 (3)	1667 (2)	6812 (1)	107 (4)	53 (2)	76 (1)	−9 (3)	−1 (1)	1 (1)
C(10)	0825 (5)	3087 (3)	6178 (1)	186 (7)	112 (4)	12 (1)	29 (5)	−4 (2)	16 (1)
				Ephedrine B					
C(1)	7636 (4)	7483 (3)	6045 (1)	126 (5)	82 (3)	8 (1)	−10 (3)	−4 (1)	−1 (1)
C(2)	8149 (5)	6304 (3)	5978 (1)	184 (7)	91 (3)	12 (1)	−12 (4)	7 (1)	−8 (1)
C(3)	9171 (6)	5971 (4)	5999 (1)	229 (8)	120 (4)	14 (1)	3 (5)	12 (2)	−15 (1)
C(4)	9669 (5)	6808 (4)	5286 (1)	170 (8)	179 (5)	10 (1)	32 (5)	1 (1)	−13 (1)
C(5)	9221 (5)	7967 (4)	5348 (1)	190 (7)	169 (5)	10 (1)	18 (6)	5 (2)	16 (1)
C(6)	8198 (4)	8315 (3)	5733 (1)	172 (6)	107 (4)	9 (1)	21 (4)	4 (1)	6 (1)
C(7)	6481 (4)	7817 (2)	6458 (1)	149 (6)	63 (2)	6 (1)	−4 (3)	−3 (1)	−1 (1)
C(8)	4466 (4)	7327 (3)	6419 (1)	149 (5)	68 (2)	7 (1)	−9 (3)	−2 (1)	−2 (1)
O(1)	6441 (3)	9060 (2)	6507 (1)	268 (6)	60 (2)	9 (1)	−31 (3)	7 (1)	3 (1)
C(9)	1685 (5)	6840 (4)	6902 (1)	159 (6)	106 (4)	11 (1)	−38 (4)	2 (1)	1 (1)
N(1)	3505 (3)	7477 (2)	6866 (1)	125 (4)	55 (2)	7 (2)	−5 (2)	−1 (1)	1 (1)
C(10)	3341 (5)	7932 (5)	6049 (1)	171 (7)	190 (6)	8 (1)	−26 (6)	−9 (1)	11 (1)
				Phosphate and Water					
P	5822 (1)	5164 (1)	7497 (1)	121 (1)	43 (1)	6 (1)	−1 (1)	−1 (1)	−1 (1)
O(2)	5632 (3)	6504 (1)	7512 (1)	223 (4)	46 (1)	8 (1)	2 (2)	−9 (1)	1 (1)
O(3)	6176 (3)	4875 (2)	6972 (1)	249 (1)	62 (2)	7 (1)	−31 (3)	8 (1)	−3 (1)
O(4)	7532 (2)	4758 (2)	7769 (1)	121 (3)	52 (1)	10 (1)	7 (2)	−3 (1)	−1 (1)
O(5)	4070 (3)	4510 (2)	7641 (1)	128 (4)	70 (2)	8 (1)	−11 (2)	−1 (1)	1 (1)
O(W)	0668 (4)	3701 (2)	7326 (1)	125 (4)	111 (2)	21 (1)	−1 (3)	5 (1)	−27 (1)

TABLE 10.

Final hydrogen-atom parameters and their estimated standard deviations. Positional parameters have been multiplied by 10^3.

ATOM	x	y	z	β(Å²)
Ephedrine A				
H(C2)	522(5)	048(3)	581(1)	6.0(1.0)
H(C3)	680(5)	077(3)	511(1)	7.2(1.0)
H(C4)	734(5)	282(3)	490(1)	7.2(1.0)
H(C5)	627(5)	442(3)	536(1)	6.5(.9)
H(C6)	499(5)	340(3)	602(1)	6.0(.9)
H(C7)	422(4)	119(2)	651(1)	2.6(.5)
H(C8)	146(4)	123(2)	616(1)	3.5(.6)
H(C9)	-167(4)	104(2)	711(1)	3.9(.6)
H(C9')	-192(7)	219(4)	672(1)	11.3(1.4)
H(C9")	-139(5)	070(3)	659(1)	5.2(.8)
H(C10)	128(4)	324(3)	587(1)	4.9(.7)
H(C10')	104(5)	373(3)	641(1)	6.7(.9)
H(C10")	-043(5)	300(3)	615(1)	6.7(1.1)
H(O1)	419(5)	245(3)	703(1)	5.1(.7)
H(N1)	089(4)	235(2)	700(1)	3.9(.6)
H(N1')	141(4)	098(3)	697(1)	5.0(.7)
Ephedrine B				
H(C2)	778(4)	568(3)	620(1)	4.7(.7)
H(C3)	962(5)	513(3)	553(1)	7.1(1.0)
H(C4)	1036(5)	650(3)	502(1)	6.1(.9)
H(C5)	958(5)	860(3)	517(1)	6.3(.9)
H(C6)	800(4)	910(2)	579(1)	2.7(.6)
H(C7)	714(3)	751(2)	673(1)	2.7(.5)
H(C8)	465(4)	649(2)	638(1)	3.4(.6)
H(C9)	122(5)	689(3)	722(1)	7.5(1.0)
H(C9')	090(6)	711(4)	670(1)	7.8(1.1)
H(C9")	189(6)	595(4)	689(1)	9.1(1.2)
H(C10)	396(6)	786(3)	575(1)	8.1(1.1)
H(C10')	316(8)	880(4)	614(2)	12.9(1.9)
H(C10")	203(6)	762(3)	603(1)	6.9(1.0)
H(O1)	628(6)	924(3)	682(1)	7.9(1.0)
H(N1)	333(5)	836(3)	695(1)	5.9(.8)
H(N1')	442(4)	717(3)	710(1)	4.5(.7)
Phosphate and Water				
HO(3)	570(6)	419(3)	696(1)	9.3(1.2)
H(W)	-013(6)	401(3)	748(1)	5.8(.9)
H(W')	168(5)	392(3)	743(1)	5.3(.9)

TABLE 11.

Structure factor table of ephedrine monohydrogen.

[illegible]

TABLE 12.

Bond distances. Standard deviations in bond lengths involving only nonhydrogen atoms are about 0.006 Å. Standard deviations in bond lengths involving hydrogen atoms are about 0.06 Å.

Bond	Ephedrine A	Ephedrine B	Bond	Ephedrine A	Ephedrine B
C(1)-C(2)	1.386 Å	1.394 Å	C(2) -H(C2)	.98 Å	1.00 Å
C(2)-C(3)	1.391	1.387	C(3) -H(C3)	1.06	1.02
C(3)-C(4)	1.370	1.369	C(4) -H(C4)	.94	.99
C(5)-C(4)	1.365	1.359	C(5) -H(C5)	.99	.93
C(5)-C(6)	1.390	1.407	C(6) -H(C6)	.92	.91
C(6)-C(1)	1.385	1.375	C(7) -H(C7)	.96	.99
C(1)-C(7)	1.514	1.519	C(8) -H(C8)	1.01	.97
C(7)-O(1)	1.417	1.411	N(1) -H(N1)	.95	1.05
C(7)-C(8)	1.545	1.537	C(1) -H(N1')	1.00	1.01
C(8)-N(1)	1.502	1.497	C(9) -H(C9)	1.05	1.00
C(8)-C(10)	1.514	1.516	C(9) -H(C9')	1.07	.88
N(1)-C(9)	1.495	1.482	C(9) -H(C9")	.96	1.02
			C(10)-H(C10)	.99	.98
			C(10)-H(C10')	1.01	1.03
			C(10)-H(C10")	.90	.98

Phosphate and Water

P-O(2)	1.519 Å	O(W)-H(OW)	.82 Å
P-O(3)	1.606	O(W)-H(OW')	.80
P-O(4)	1.526	O(3)-H(O3)	.84
P-O(5)	1.507		

TABLE 13.

Bond angles. Standard deviations in bond angles involving only nonhydrogen atoms are about 0.3°. Standard deviations in bond angles involving hydrogen atoms are about 4°.

	Ephedrine A	Ephedrine B		Ephedrine A	Ephedrine B
C(6)–C(1)–C(2)	118.8	118.8	H(C6)–C(6)–C(1)	117	120
C(7)–C(1)–C(6)	122.3	121.8	H(O1)–O(1)–C(7)	107	108
C(7)–C(1)–C(2)	119.0	119.5	H(C7)–C(7)–C(1)	105	108
C(1)–C(2)–C(3)	120.7	120.7	H(C7)–C(7)–O(1)	112	106
C(2)–C(3)–C(4)	119.7	119.6	H(C7)–C(7)–C(8)	107	112
C(3)–C(4)–C(5)	120.2	120.9	H(C8)–C(8)–C(7)	104	109
C(4)–C(5)–C(6)	120.6	119.9	H(C8)–C(8)–C(10)	115	115
C(5)–C(6)–C(1)	120.0	120.1	H(C8)–C(8)–N(1)	106	107
O(1)–C(7)–C(1)	111.2	109.8	H(C10)–C(10)–C(8)	111	112
C(8)–C(7)–O(1)	111.2	110.3	H(C10′)–C(10)–C(8)	108	108
C(8)–C(7)–C(1)	111.3	110.6	H(C10′′)–C(10)–C(8)	109	112
C(7)–3(8)–3(10)	114.6	112.5	H(N1)–N(1)–C(8)	109	113
C(7)–C(8)–N(1)	107.1	108.4	H(N1)–N(1)–HN(1′)	110	104
C(10)–C(8)–N(1)	109.7	110.3	H(N1)–N(1)–C(9)	105	110
C(8)–N(1)–C(9)	113.8	113.9	H(N1′)–N(1)–C(8)	114	106
H(C2)–C(2)–C(1)	120	120	H(N1′)–N(1)–C(9)	105	110
H(C2)–C(2)–C(3)	119	119	H(C9)–C(9)–N(1)	109	109
H(C3)–C(3)–C(2)	119	125	H(C9′)–C(9)–N(1)	105	110
H(C3)–C(3)–C(4)	121	115	H(C9′′)–C(9)–N(1)	108	111
H(C4)–C(4)–C(3)	122	115	H(C10)–C(10)–H(ClO′)	106	111
H(C4)–C(4)–C(5)	118	124	H(C10)–C(10)–H(ClO′′)	105	110
H(C5)–C(5)–C(4)	126	127	H(C10′)–C(10)–H(C10′′)	117	103
H(C5)–C(5)–C(6)	114	113	H(C9)–C(9)–H(C9′)	111	115
H(C6)–C(6)–C(5)	123	119	H(C9)–C(9)–H(C9′′)	103	99
			H(C9′)–C(9)–H(C9′′)	121	113

Phosphate and Water

O(2)–P–O(3)	104.1	O(3)–P–O(5)	107.6
O(2)–P–O(4)	110.7	O(4)–P–O(5)	111.1
O(2)–P–O(5)	114.0	H(O3)–O(3)–P	100
O(3)–P–O(4)	108.9	H(W)–O(W)–H(W′)	106

TABLE 14.

Hydrogen-bond distances and angles.

DONOR ATOM	HYDROGEN ATOM	ACCEPTOR ATOM	DISTANCES (Å) DONOR-ACCEPTOR	ACCEPTOR-HYDROGEN	DONOR-HYDROGEN-ACCEPTOR ANGLE (DEGREES)
MOLECULE A					
O(1)	H(O1)	O(2) (I)	2.61 Å	1.72 Å	166°
O(3)	H(O3)	O(1)	2.74	1.93	163
N(1)	H(N1)	O(W)	2.75	1.59	170
N(1)	H(N1')	O(4) (I)	2.75	1.75	179
MOLECULE B					
O(1)	H(O1)	O(5) (II)	2.60	1.64	178
N(1)	H(N1)	O(4) (II)	2.89	1.88	163
N(1)	H(N1')	O(2)	2.64	1.67	170
PHOSPHATE AND WATER					
O(W)	H(OW')	O(5) (III)	2.74	1.93	175
O(W)	H(OW)	O(4) (III)	2.85	2.06	170

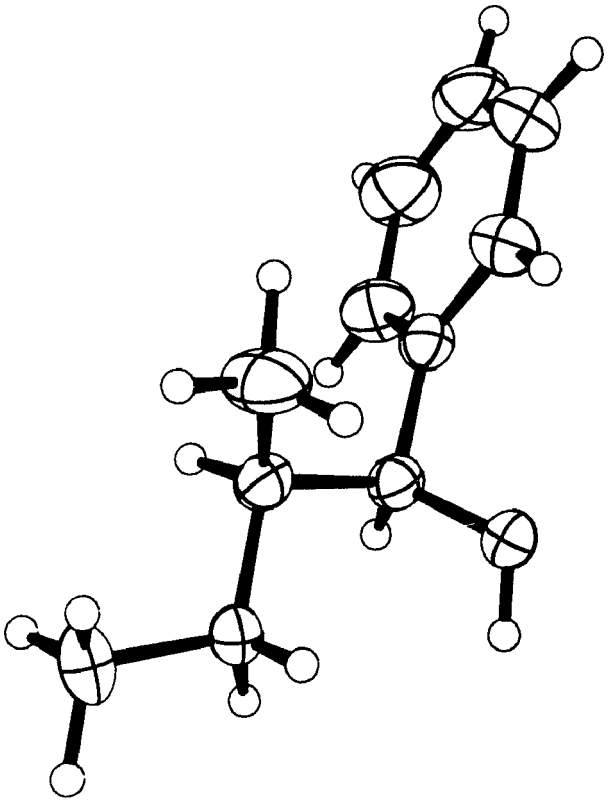
Symmetry Codes: (I): 1 - x, y - 1/2, 3/2 - z

(II): 1 - x, y + 1/2, 3/2 - z

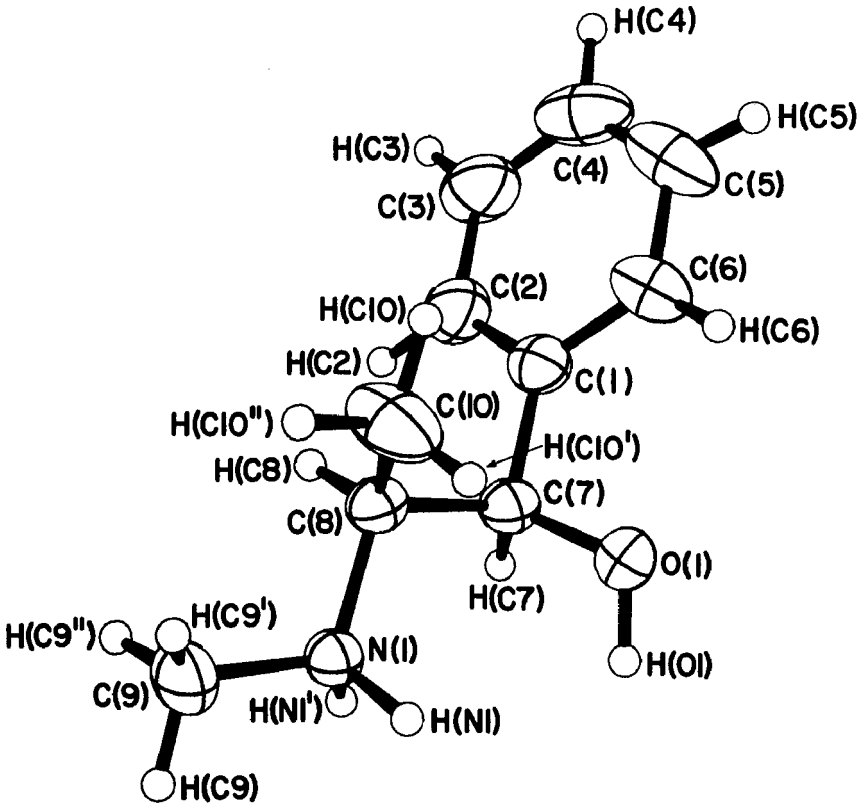
(III): x - 1, y, z

Figure 6.

Conformations of the two ephedrine cations (labelled molecule A and molecule B). Heavy atoms are represented by ellipsoids, defined by the principal axes of thermal vibration, and scaled to include 50% probability. Hydrogen atoms are represented by spheres with 0.1 Å radius.



MOLECULE A



MOLECULE B

Figure 7.

The crystal system viewed down the a axis. The thin lines represent hydrogen bonds.

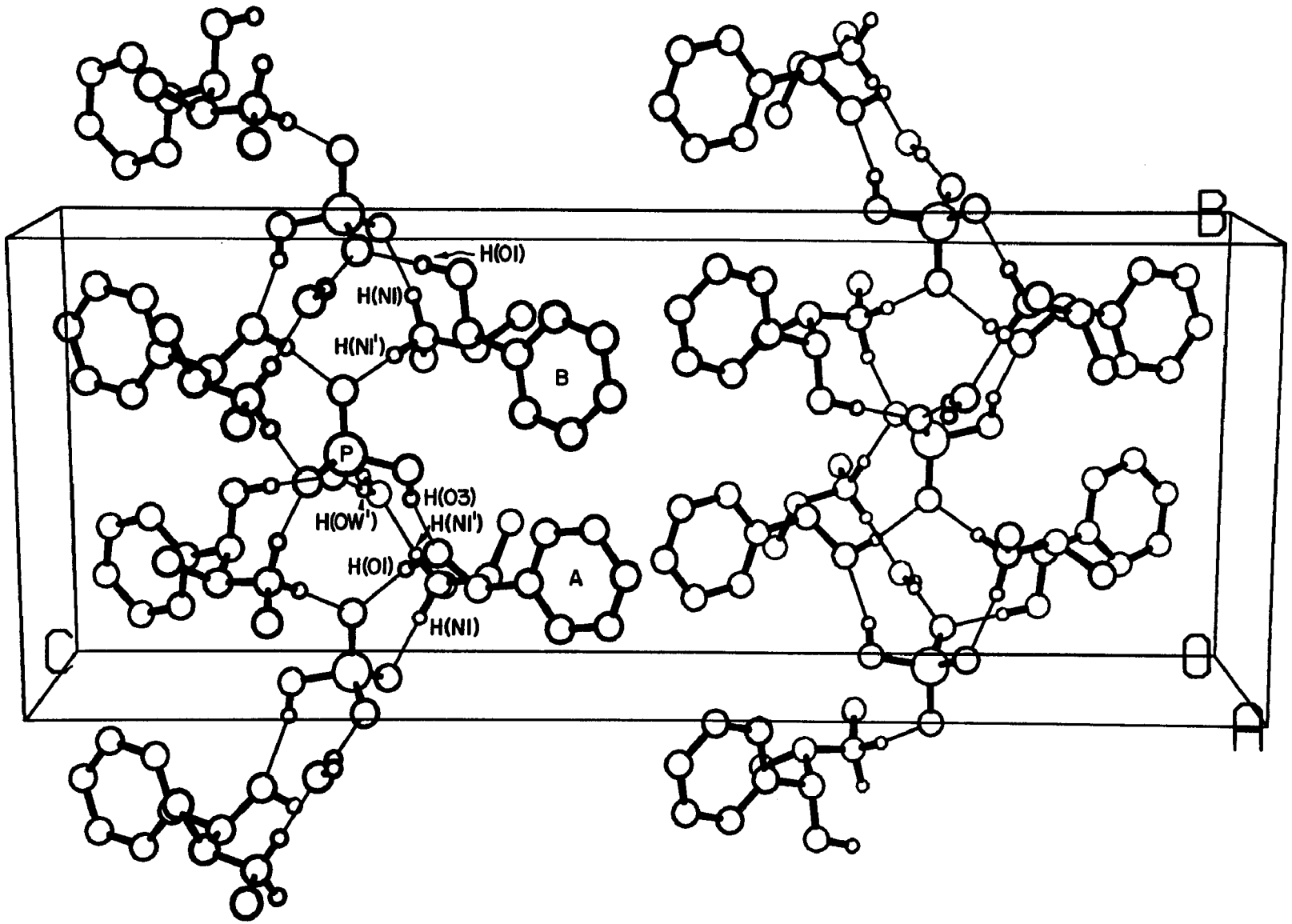
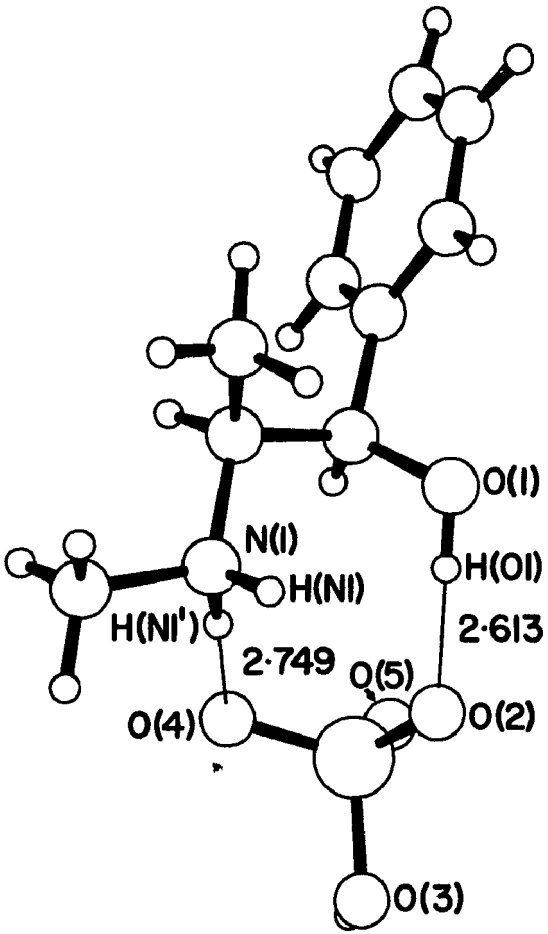
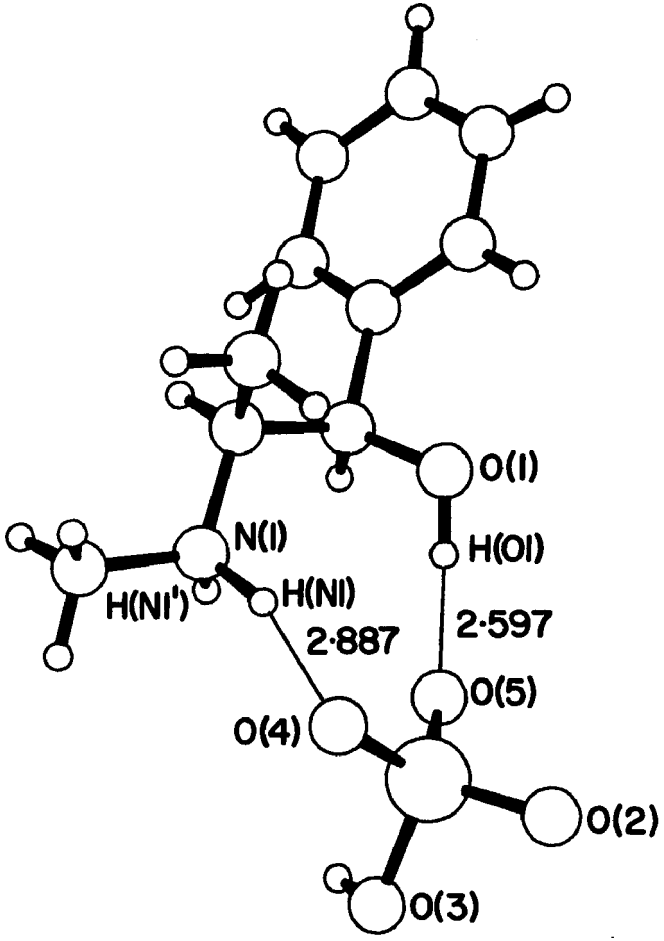

H(O1)
H(N1)
H(N1')
B
P
H(O3)
H(OW')
H(N1')
H(O1)
A
H(N1)
B
C
A

Figure 8.

Hydrogen bonding between the ephedrine cations and the phosphate anion. Hydrogen bonds are represented by thin lines, and donor-acceptor distances (Å) are given.



MOLECULE A



MOLECULE B

III. Ethanolamine Monohydrogen Phosphate

A. Experimental

Small thin plates of ethanolamine phosphate were grown from an aqueous solution with a 2:1 ratio of ethanolamine and phosphoric acid. Weissenberg and oscillation photographs showed the plates to be monoclinic with systematic extinctions 0k0 with k odd and h0ℓ with h odd. This indicated space group $P2_1/c$; however, the h0ℓ with h odd were systematically, extremely weak from a pseudo a glide perpendicular to the b axis. A crystal with approximate dimensions of .3, .3, and .1 mm was mounted on the diffractometer with its a axis along the ϕ axis of the diffractometer. Approximate cell parameters for use in collection of intensity data were calculated by a least-squares analysis of the angular settings for twelve low-angle CuKα(λ = 1.5418 Å) reflections. Crystal data are listed in Table 15.

Intensity data were collected with the diffractometer, by using a scintillation counter, nickel-filtered copper radiation, and θ-2θ scanning technique. Measurements were made for the 3319 reflections with $2\theta < 128°$. Intensity values were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus an

additional term $(0.03S)^2$, S being the scan counts. The intensities and their variances were corrected for Lorentz and polarization factors, and absorption corrections were applied by using the program ORABS (37). Structure factors and variances were placed on an approximately absolute scale by means of a Wilson plot (39). Immediately after data collection, accurate values for the cell parameters were determined by an analysis of 2θ values for high-angle reflections ($\lambda = 1.54051$ Å) measured with the diffractometer.

Trial coordinates for the two phosphorous and for the eight oxygen atoms of the phosphate groups were obtained by direct methods, with the computer program MULTAN (19). The other nonhydrogen atoms were located in a Fourier map that was calculated with phase angles derived from the ten heavy atoms of the two phosphate groups. The trial structure was refined by use of a modified version of the full matrix least-squares program ORFLS (4). The quantity minimized was $\Sigma \underline{w}(Fo^2-(1/k)Fc^2)^2$, where k is a scale factor and weight $\underline{w}$ is equal to $(1/\sigma(Fo^2))^2$. All reflections were included in the refinements. Atomic scattering factors for the nonhydrogen atoms were taken from International Tables for X-Ray Crystallography (42); anomalous dispersion corrections for these atoms were those of Cromer and Liberman (7). The hydrogen-atom scattering factors were taken from Stewart, Davidson, and Simpson (33).

Hydrogen atoms bonded to carbon were calculated by assuming tetrahedral bonds; hydrogen atoms bonded to oxygen and nitrogen were calculated by assuming linear hydrogen bonds. The last cycles of refinement included all positional parameters and anisotropic temperature parameters for the nonhydrogen atoms. Because of the limited core-storage capacity of the computer it was impracticable to refine all parameters simultaneously; consequently, the parameters were divided into two blocks each consisting of two ethanolamines and one phosphate. The blocks were refined in successive cycles. The final R index ($\Sigma||Fo|-|Fc||/\Sigma|Fo|$) for all reflections, including those with negative intensities, is 11.09; the goodness-of-fit $(\Sigma(1/\sigma^2 Fo^2))(Fo^2-Fc^2/k^2)/(m-s))^{1/2}$, where m is the number of reflections used and s is the number of parameters refined, is 2.18*. During the last cycle of refinement no parameter shifted more than one-fifth of its standard deviation. A final difference Fourier map revealed no peaks or troughs exceeding 1 e/Å^3 in magnitude.

B. Results

Table 16 lists the final heavy atom parameters and their estimated standard deviations; the average estimated standard deviation in these positional parameters is .005 Å. Table 17 gives the hydrogen-atom parameters

---

*The final R index for all reflections with intensities greater than one standard deviation is 9.77.

which were calculated on the basis of the expected covalent and hydrogen-bonding angles. Table 20 lists the observed and calculated structure factors.

Bond lengths are given in Table 18 and bond angles are listed in Table 19. Crystal-packing and hydrogen-bonding schemes are shown in Figure 9. Three of the four crystallography independent ethanolamines assume the gauche conformation. The specific phosphate-ethanolamine interaction, in which one ethanolamine chelates one phosphate anion similar to that found in ephedrine monohydrogen phosphate, is shown in Figure 10. Phosphate anions seem to form strong hydrogen bonds to other phosphate anions without decreasing their affinity for the ethanolamine moiety.

TABLE 15. Crystal data for ethanolamine monohydrogen phosphate.

Stoichiometry	$(C_2H_8O\ N)_4 \cdot (HPO_4)_2$
Z	4
Space Group	$P2_1/c$
a	9.128(3) Å
b	10.988(4)
c	22.354(10)
β	115.78(3)°
ρ (calculated)	1.424 g. cm^{-3}
ρ (observed	1.44 g. cm^{-3}
μ	24.9 cm^{-1}

(The unit-cell parameters were measured at 25 ± 3°C. The reported standard deviations are five times those obtained from the least-squares analysis. The density was measured by flotation in a mixture of benzene and ethylene dibromide.)

TABLE 16.

Final heavy-atom parameters and their estimated standard deviations. All values have been multiplied by 10^4. Temperature factors are in the form $T = \exp(-\beta_{11}h^2-\beta_{22}k^2-\beta_{33}\ell^2-2\beta_{12}hk-2\beta_{13}h\ell-2\beta_{23}k\ell)$. Final value of the isotropic extinction parameter (g) is 0.278.

ATOM	X	Y	Z	B11(OR B)	B22	B33	B12	B13	B23
P	1014(2)	3717(1)	2681(1)	63(2)	42(1)	19(1)	2(2)	16(1)	-1(1)
O1	1104(4)	2345(3)	2738(2)	77(6)	49(4)	19(1)	0(4)	18(2)	-1(2)
O2	-807(4)	4124(3)	2239(2)	59(6)	67(4)	24(1)	3(4)	16(2)	6(2)
O3	1579(5)	4324(3)	3359(2)	129(8)	59(4)	21(1)	2(4)	14(3)	-10(2)
O4	1938(5)	4215(3)	2304(2)	103(7)	57(4)	31(1)	9(4)	38(3)	11(2)
P'	5972(2)	3375(1)	2673(1)	59(2)	42(1)	20(1)	0(2)	15(1)	-1(1)
O1'	6081(4)	4749(3)	2716(2)	84(6)	48(4)	20(1)	1(4)	20(2)	-2(2)
O2'	4180(4)	2979(3)	2202(2)	60(6)	65(4)	22(1)	-2(4)	11(2)	-10(2)
O3'	6456(5)	2811(4)	3351(2)	166(8)	61(4)	20(1)	-11(5)	11(3)	6(2)
O4'	6970(4)	2839(3)	2338(2)	82(7)	64(4)	36(2)	-15(4)	39(3)	-15(2)
C1(1)	387(9)	1610(6)	1055(3)	173(14)	96(8)	21(2)	8(8)	31(5)	11(3)
N1(1)	810(5)	932(4)	1669(2)	94(8)	61(5)	16(1)	-2(5)	16(3)	8(2)
C2(1)	-1167(12)	2284(8)	856(4)	428(26)	134(11)	14(2)	140(13)	16(6)	23(4)
O5(1)	-1797(9)	2718(6)	234(4)	393(18)	152(8)	56(3)	43(10)	13(6)	12(4)
C1(2)	5548(10)	5479(7)	1030(4)	195(16)	139(10)	26(2)	-40(10)	33(5)	-18(4)
N1(2)	5810(6)	6146(4)	1638(2)	114(9)	61(5)	17(2)	-7(5)	14(3)	-2(2)
C2(2)	4281(14)	4650(9)	787(4)	322(24)	174(13)	25(3)	-97(14)	34(7)	-33(5)
O5(2)	2851(10)	5060(10)	699(6)	226(17)	513(22)	166(7)	-140(15)	105(9)	-219(11)
C1(3)	283(8)	1156(6)	4142(3)	132(12)	113(8)	17(2)	-5(8)	14(4)	11(3)
N1(3)	-631(6)	1303(4)	3425(2)	108(9)	58(5)	14(1)	-9(5)	9(3)	5(2)
C2(3)	1061(9)	2349(7)	4475(3)	168(14)	125(9)	16(2)	-15(9)	15(4)	-9(3)
O5(3)	2412(5)	2655(4)	4371(2)	123(8)	94(5)	22(1)	-3(5)	6(3)	5(2)
C1(4)	5426(8)	5997(6)	4105(3)	148(13)	71(7)	20(2)	-5(7)	15(4)	-8(3)
N1(4)	4383(5)	5781(4)	3388(2)	96(8)	53(5)	19(2)	6(5)	18(3)	3(2)
C2(4)	6118(9)	4849(7)	4490(3)	178(15)	106(8)	21(2)	13(9)	19(5)	5(4)
O5(4)	7391(5)	4343(4)	4375(2)	137(9)	117(6)	24(2)	37(5)	10(3)	-7(2)

TABLE 17.

Final hydrogen-atom parameters. Positional parameters have been multiplied by 10^3.

Atom	X	Y	Z
H1	-164	364	228
H1'	333	345	224
H2(1)	-260	160	20
H3(1)	92	142	205
H4(1)	0	38	167
H5(1)	185	54	188
H6(1)	124	220	110
H7(1)	33	107	70
H8(1)	-109	290	117
H9(1)	-199	168	89
H2(2)	240	640	60
H3(2)	505	672	165
H4(2)	591	567	201
H5(2)	685	655	185
H6(2)	660	513	110
H7(2)	532	609	68
H8(2)	454	394	113
H9(2)	426	416	41
H2(3)	212	325	401
H3(3)	-108	58	317
H4(3)	-5	165	319
H5(3)	-140	179	307
H6(3)	-38	83	433
H7(3)	118	58	424
H8(3)	21	298	429
H9(3)	134	232	494
H2(4)	704	378	400
H3(3)	-108	58	317
H4(3)	-5	165	319
H5(3)	-140	179	307
H6(4)	633	652	414
H7(4)	483	645	429
H8(4)	651	501	496
H9(4)	523	427	438

TABLE 18.

Bond distances. Standard deviations in bond angles involving nonhydrogen atoms are about .007 Å.

Bond	Distance
P(1)-O(2)	1.513
P(1)-O(2)	1.583
P(1)-O(3)	1.528
P(1)-O(4)	1.530
P(1)'-O(1)'	1.511
P(1)'-O(2)'	1.575
P(1)'-O(3)'	1.514
P(1)'-O(4)'	1.523
N1(1)-C1(1)	1.459
C1(1)-C2(1)	1.487
C2(1)-O5(1)	1.340
N1(2)-C1(2)	1.468
C1(2)-C2(2)	1.383
C2(2)-O5(2)	1.314
N1(3)-C1(3)	1.459
C1(3)-C2(3)	1.521
C2(3)-O5(3)	1.393
N1(4)-C1(4)	1.484
C1(4)-C2(4)	1.503
C2(4)-O5(4)	1.410

TABLE 19.

Bond angles. Standard deviations in bond angles involving nonhydrogen atom are about .3 degrees.

Bond	Angle
O(1)-P(1)-O(2)	110.0
O(1)-P(1)-O(3)	111.8
O(1)-P(1)-O(4)	112.4
O(2)-P(1)-O(3)	107.8
O(2)-P(1)-O(4)	103.4
O(3)-P(1)-O(4)	111.1
O(1)'-P(1)'-O(2)'	109.7
O(1)'-P(1)'-O(3)'	110.9
O(1)'-P(1)'-O(4)'	112.4
O(2)'-P(1)'-O(3)'	108.9
O(2)'-P(1)'-O(4)'	103.9
O(3)'-P(1)'-O(4)'	110.9
N1(1)-C1(1)-C2(1)	111.3
C1(1)-C2(1)-O5(1)	114.2
N1(2)-C1(2)-C2(2)	117.7
C1(2)-C2(2)-O5(2)	116.0
N1(3)-C1(3)-C2(3)	111.4
C1(3)-C2(3)-O5(3)	112.7
N1(4)-C1(4)-C2(4)	113.4
C1(4)-C2(4)-O5(4)	113.6

Figure 9.

The crystal structure viewed in stereo down the b axis. Dashed lines represent hydrogen bonds.

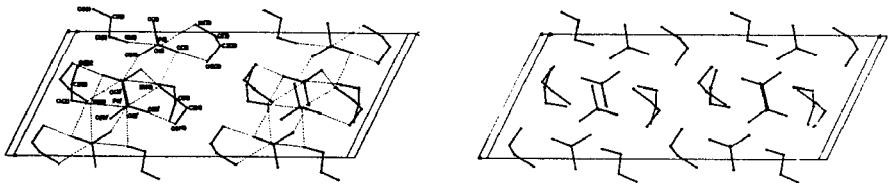
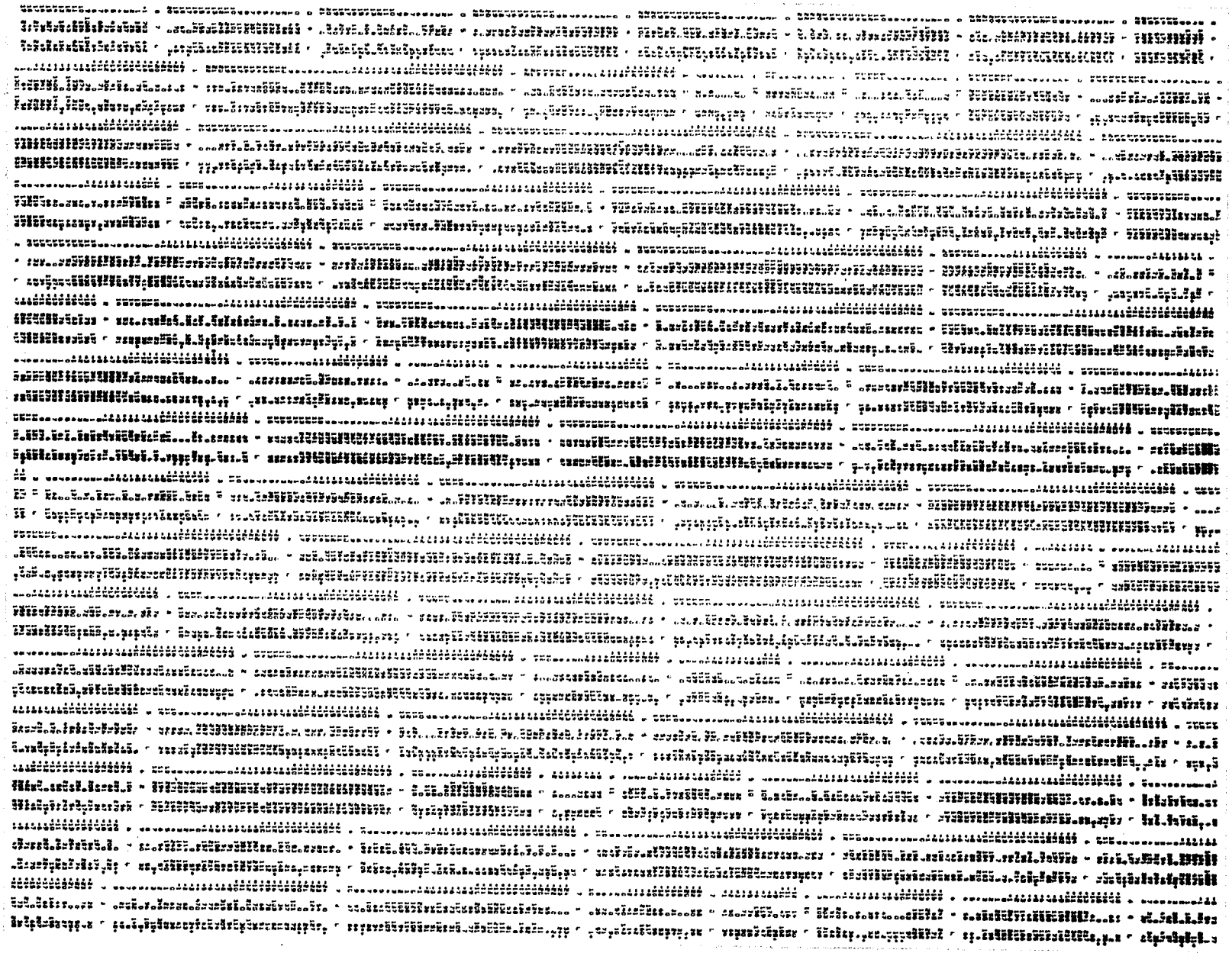


TABLE 20.

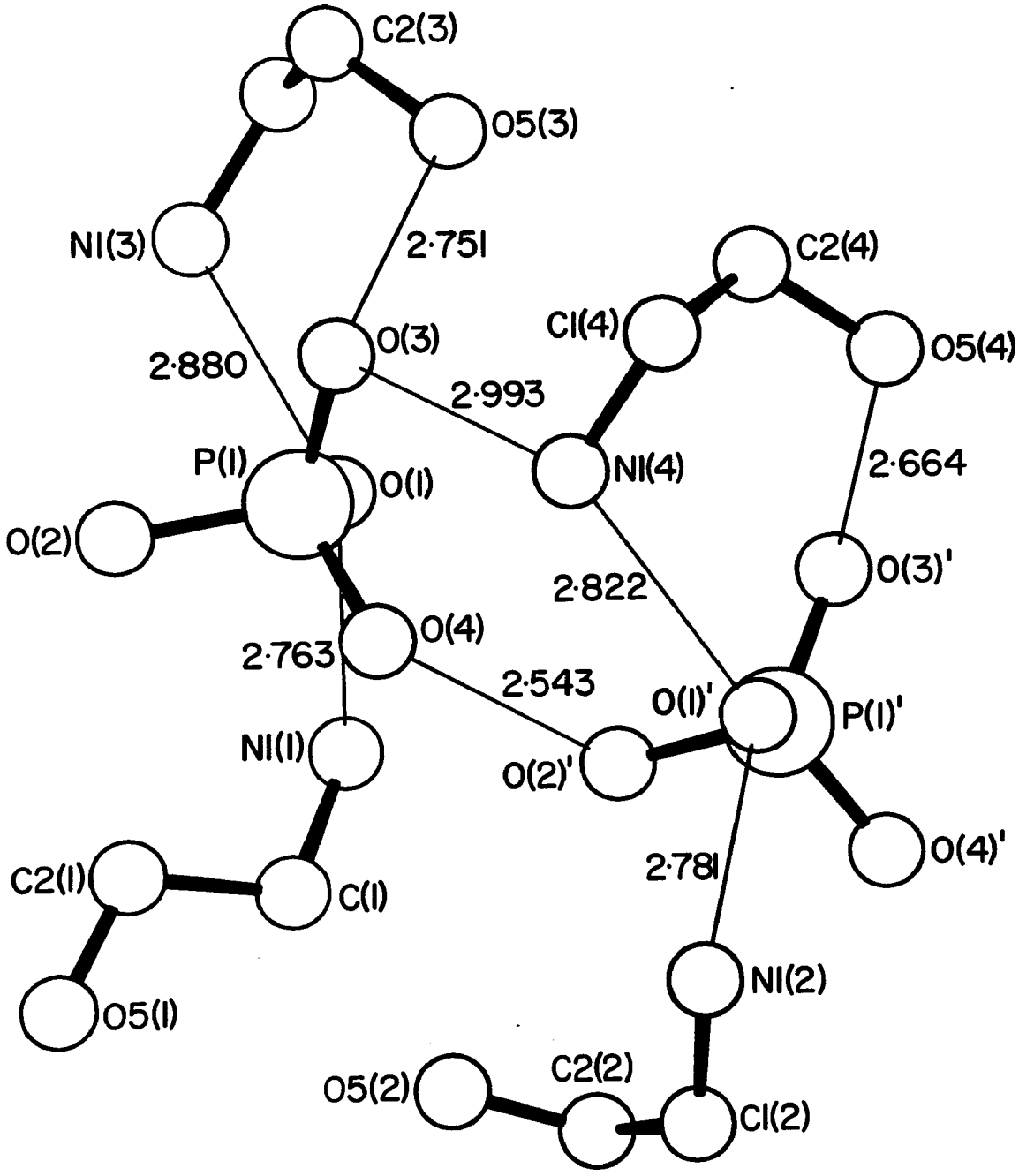
Observed and calculated structure factors for ethanolamine monohydrogen phosphate are shown on the following two pages.



[illegible]

Figure 10.

The asymmetric unit shows the four ethanolamines and two phosphate anions with hydrogen bonds depicted by the thin lines. Hydrogen-bond lengths representing the distance from the donor to the acceptor are shown.


C2(3)
O5(3)
Ni(3)
2·751
O(3)
2·880
2·993
P(1)
O(1)
O(2)
O(4)
2·763
2·543
Ni(1)
C2(1)
C(1)
O5(1)
C2(4)
Cl(4)
O5(4)
Ni(4)
2·664
O(3)'
2·822
O(1)'
P(1)'
O(2)'
O(4)'
2·781
Ni(2)
O5(2)
C2(2)
Cl(2)

DISCUSSION

I. Conformational Properties of Ethanolamines

As part of this series of structural studies, I examined the conformations of several crystallographically-independent ethanolamines that were complexed with phosphate anions. These conformations were compared with those of ethanolamines in other published crystal structures which contained no phosphate anions. Conformational data for twelve ethanolamine molecules, which represent a variety of solid-state environments, are available for comparison with my structural results for ethanolamine phosphates. Pertinent conformational angles for these ethanolamines are depicted in Figure 11.

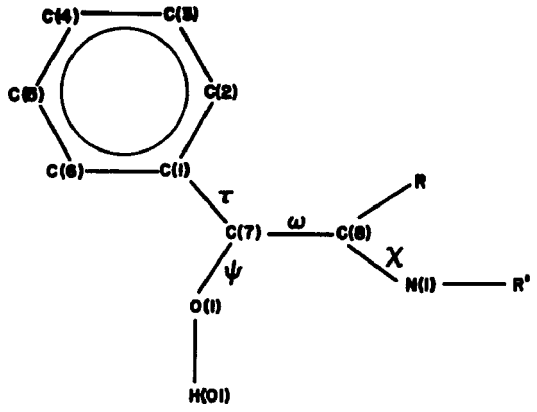
The conformations of these ethanolamines are conveniently represented by four torsion angles, which I designate and define as follows: τ describes the conformation about the C(1)-C(7) bond and is defined by atoms C(6)-C(1)-C(7)-O(1) (17); ω describes the conformation about the C(7)-C(8) bond and is defined by atoms O(1)-C(7)-C(8)-N(1); χ describes the conformation about the N(1)-C(8) bond and is defined by atoms R'-N(1)-C(8)-C(7); and ψ describes the conformation about

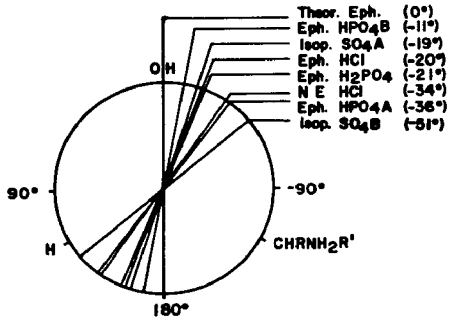
the O(1)-C(7) bond and is defined by HO(1)-O(1)-C(7)-C(8). The values of the torsion angles are assigned according to the convention of Klyne and Prelog (17). This convention defines a torsion angle by using four atoms designated as A-B-C-D, in the following manner: if a system of four atoms, A-B-C-D, is projected on to a plane normal to bond B-C, the angle between the projection of A-B and the projection of C-D is described as the torsion angle of A and D about bond B-C; the torsion angle is written in full as (A-B-C-D); in the eclipsed conformation in which the projections of A-B and C-D coincide, θ is given the value 0°; a torsion angle is considered positive ($+\theta$) or negative ($-\theta$), if on viewing the system along the central bond in the direction B C, the bond to the front atom A requires rotation to the right or left, respectively, in order that it can eclipse the bond to the rear atom D.

As shown in Figure 11, the conformational angles for the ethanolamines are confined to relatively narrow ranges. The clustering of these four conformational angles within specified ranges suggests that restraints are imposed on the rotation around these single bonds, thus causing certain conformations to be preferred. More recent crystallographic studies (8) by Dangoumau on similar ethanolamine compounds support this hypothesis. The conformational angle ω defines the spatial arrangement of the hydroxyl and amino groups of the ethanolamine moiety.

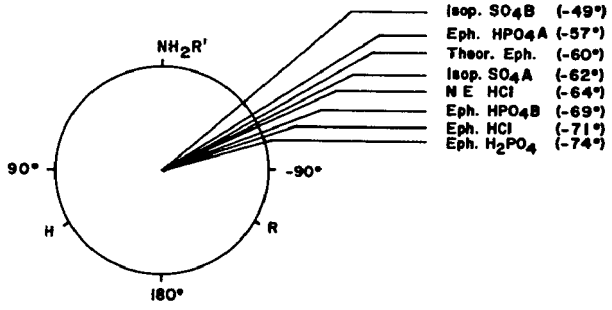
Figure 11.

Conformational maps depicting the torsion angles in phenylethanolamines. Each torsion angle (17) is described by four atoms, A-B-C-D, and is defined as the angle that A-B makes with respect to C-D when viewed down the B-C bond. The angle is zero if A-B and C-D are eclipsed, and is positive if A-B must be rotated clockwise to eclipse C-D. Each drawing shows the trace of A-B as viewed down B-C. Abbreviations for the compounds are: Theor. Eph., molecular orbital calculation for ephedrine (16); Eph. HPO_4A and Eph. HPO_4B, the A and B molecules in the crystal structure of ephedrine monohydrogen phosphate monohydrate; Isop. SO_4A and Isop. SO_4B, the A and B molecules in the crystal structure of isoproterenol sulphate (20); Eph. H_2PO_4, crystal structure of ephedrine dihydrogen phosphate; N E HCl, crystal structure of norepinephrine hydrochloride (3).

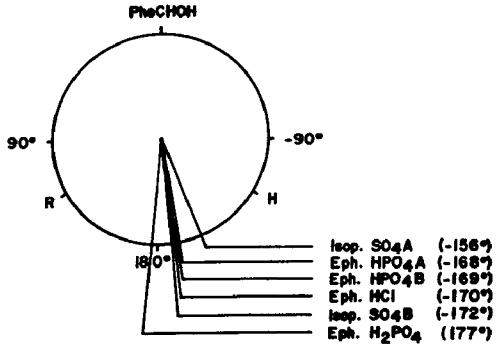




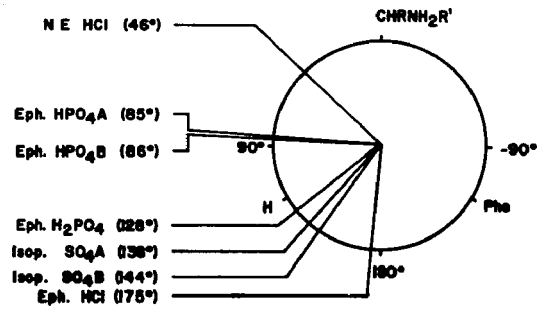
τ(C(6)-C(1)-C(7)-O(1))



ω(O(1)-C(7)-C(8)-N(1))



χ(R'-N(1)-C(8)-C(7))



ψ(H(O1)-O(1)-C(7)-C(8))

In nearly all the structures I examined, this torsion angle is such that the amino group is gauche to the hydroxyl group. This finding is of particular significance because it implies that the preferred solid-state conformation of ethanolamines is exactly the one that permits strong hydrogen bonding to phosphate ions. This preference for the gauche conformation is not merely a consequence of solid-state forces, as evidenced by solution (16,25) and gas phase spectroscopic (23) studies, which demonstrate that the gauche conformation is the most stable one for various ethanolamines.

Though the reason for this preference for the gauche conformation is not clearly understood, an intramolecular hydrogen bond between the amino and hydroxyl groups has been postulated (25). We have examined the hydroxyl-amino contacts within the ethanolamines that are included in Figure 11, and, with the possible exception of the B molecule in the crystal structure of isoproterenol sulphate, we have found no examples of intramolecular hydrogen bonding between the hydroxyl and amino group. The possible existence of weak, stabilizing interactions between the amino hydrogen and the hydroxyl oxygen atoms apparently is not attributable to hydrogen bonding, as evidenced by the long N-H--O distances and the nonlinear N-H--O angles. It is noteworthy that acetylcholine and several related compounds, which have the R_3^+N-C-C-O grouping, have been found to assume a gauche conformation about the C-C bond (31), even though,

intramolecular N--O hydrogen bonding is not possible. This preference for the gauche conformation in acetylcholine has been attributed to an electrostatic attraction between the oxygen and the cationic nitrogen atoms (31), and this same interaction may govern the torsion angles about the C(7)-C(8) bonds of ethanolamines (ω = -75, -74, -53, -166). Regardless of the mechanism involved, the gauche conformation is obviously the most stable. Bulky substituents, such as phenyl groups, which are attached to C(7) in phenylethanolamines, might be expected to be trans to the amino group for steric reasons, thus forcing the amino group to lie gauche to the hydroxyl moiety. However, this cannot be the only explanation for the observed conformations of the phenylethanolamines since the preferred conformation of ethanolamines, without bulky groups attached to C(7), also has the amino and hydroxyl groups gauche to each other. This preference of the hydroxyl group to be gauche to the amino group in ethanolamines probably has important biological implications particularly as it relates to the propensity of ethanolamines for phosphates.

II. Phosphate-Phosphate Interactions

Extensive hydrogen bonding between the phosphate anions is observed in the crystal structure of both ephedrine dihydrogen phosphate and ethanolamine monohydrogen phosphate. In the ephedrine dihydrogen phosphate structure, the phosphate anions hydrogen bond to each other to form continuous phosphate sheets. Each of these phosphate anions forms four exceptionally short hydrogen bonds (with lengths of either 2.55 Å or 2.63 Å) to three other phosphate anions. In the ethanolamine monohydrogen phosphate structure the phosphate anion forms strong bonds to two other phosphate anions. Both the mono- and dihydrogen phosphate anions are particularly suited for the forming of strong hydrogen bonds because of their excellent hydrogen-bond donor sites (acidic protons) and hydrogen-bond acceptor sites (negatively charged oxygens), and these donors and acceptors are joined by a polarizable pi electron system. It is not surprising that the literature contains numerous examples of crystal structures in which the phosphate anions are joined by short (<2.65 Å) hydrogen bonds (1,11,12,26,27,36), some of which appear to be symmetric (<2.5 Å) (24). In the crystal structures that have been examined to date, dihydrogen phosphate anions always form at least two hydrogen bonds to other phosphates and, if the two hydrogen

bonds extend to a single phosphate anion, an exceptionally stable phosphate dimer is formed (Figure 3). Hydrogen bonding between phosphates is an important phenomenon not only in the solid-state, but in aqueous solution as well (30). In view of the widespread occurrence of phosphate-phosphate hydrogen bonding in various *in vitro* systems, it seems likely that interactions of this type are important in biological systems.

Though interactions between the various mineral components of calcified tissues have been studied extensively, the principal emphasis has been placed on the importance of coulombic factors, whereby cations interact with anions to generate mineral phases. The crystallographic results for ephedrine dihydrogen phosphate and ethanolamine monohydrogen phosphate suggest that phosphate-phosphate interactions may also serve as an important mechanism for the aggregation of phosphate minerals in biological systems.

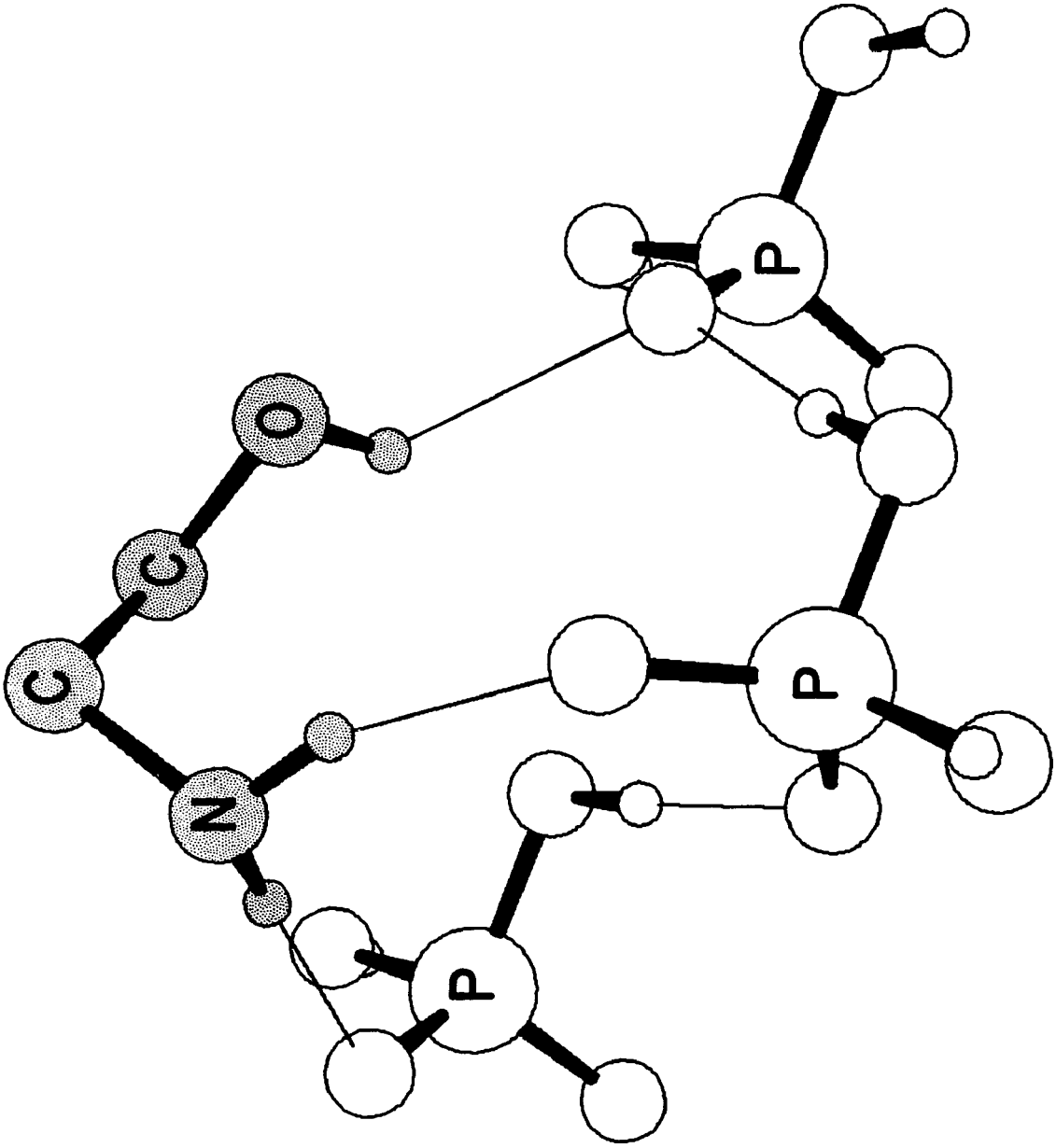
III. Interactions Between Phosphates and Ethanolamines

From the previous discussion, it is apparent that the most stable conformation of ethanolamines is one in which the amino group is situated gauche to the hydroxyl group. It is clear from these crystallographic results that ethanolamines in this gauche conformation can, by using the amino and hydroxyl groups in concert, hydrogen-bond strongly to phosphate ions. These hydrogen-bonded interactions can occur in two different ways: (1) the ethanolamine can hydrogen bond to hydrogen-bonded aggregates of phosphate anions, or (2) they can hydrogen bond to single phosphate ions. Both types of interaction are probably involved in biological processes.

The ethanolamine interaction with phosphate aggregates is an important feature of the ephedrine dihydrogen phosphate structure. As indicated in Figure 12, the ethanolamine moiety, which is in the gauche conformation, possesses three hydrogen-bond donor sites that are suitably arranged to form hydrogen bonds to phosphate ions of the phosphate aggregates. Two of the donor sites are provided by the amino group, and each of these sites hydrogen-bonds to a phosphate ion in the aggregate. The third donor site is supplied

Figure 12.

The ethanolamine moiety of the ephedrine from the crystal structure, ephedrine dihydrogen phosphate, is shown to hydrogen bond to three phosphate anions which hydrogen bond to themselves.


O
C
C
N
P
P
P

by the hydroxyl group, which hydrogen-bonds to an additional phosphate ion in the aggregate. Consequently, the ethanolamine moiety is directly hydrogen-bonded to three different phosphate ions, which are, in turn, linked to other phosphate ions within the phosphate aggregates. These two features - the hydrogen-bonding between phosphate ions to produce phosphate aggregates, and the subsequent binding of these phosphate aggregates to ethanolamines - could serve as an early step in the mineralization of bone collagen. Through interactions of this type, single isolated hydroxylysine residues of collagen could bind large arrays of phosphate ions. Such phosphate-hydroxylysine complexes might serve as key sites for the nucleation of mineral growth within collagen matrices.

Interactions of ethanolamines with single phosphate ions are outstanding features in the crystal structures of ephedrine monohydrogen phosphate and ethanolamine monohydrogen phosphate. These two structures contain a total of six crystallographically independent ethanolamine moieties, five of which are in the preferred gauche conformation (two from the ephedrine structure and three from the ethanolamine structure). Two of three gauche ethanolamine moieties in the ethanolamine monohydrogen phosphate structure (Figure 10) and both of the ethanolamine moieties in the ephedrine monohydrogen phosphate structure (Figure 8) hydrogen bond to single phosphate groups by using the amino

group in concert with hydroxyl group. Since this hydrogen-bonded interaction was observed for ethanolamine moieties in five different crystalline environments, it is obvious that this mode of association is particularly favorable, and is not simply a consequence of crystal-packing forces.

A significant factor in the hydrogen-bonding between an ethanolamine moiety and a single phosphate group is the role that the hydroxyl group plays. As judged by hydrogen-bond lengths, the strongest hydrogen-bonding to the phosphate anion involves the hydroxyl group, rather than the cationic amino group. Thus it is likely that ethanolamine-phosphate interactions are much stronger than interactions between simple amines and phosphate ions. In fact, this conclusion is supported by other spectroscopic studies, which indicate that phenylethylamines (phenyl-ethanolamines without the hydroxyl group) do not bind to the phosphate moiety of ATP, whereas phenylethanolamines do (38).

Interaction between the ethanolamine moieties of hydroxylysine residues and single phosphate ions could provide a second major mechanism for binding phosphate ions to bone collagen. At physiological pH levels, ortho-phosphate exists primarily as a mixture of $H_2PO_4^-$ and $HPO_4^=$ ions. As discussed previously, $H_2PO_4^-$ ions show a remarkable tendency to aggregate into hydrogen-bonded arrays, with which the ethanolamine moieties of hydroxylysine

should be able to interact strongly. Alternatively, the hydroxylysine residues could bind to single phosphate anions, through interactions similar to those depicted in Figures 8 and 11. This second mode of interaction appears to be particularly favorable for $HPO_4^=$ anions, which display less tendency to aggregate than do $H_2PO_4^-$ ions. Thus hydroxylysine residues could serve as ideal binding sites for both monohydrogen phosphate anions and dihydrogen phosphate ions. Considering the importance of the hydroxyl group in the formation of phosphate-ethanolamine complexes, one might reasonably expect that phosphates would have a much higher affinity for hydroxylysine than for lysine or other cationic sites of collagen.

Interactions like those depicted in Figures 8 and 11 are also of likely importance in controlling biological interactions between ATP and phenylethanolamines. In biological systems, epinephrine and norepinephrine, two phenylethanolamines that function as hormones and neurotransmitters, when released from storage vesicles diffuse to appropriate receptor sites (2,35). Various studies have indicated that these phenylethanolamines are complexed with ATP in storage vesicles (13,21,22,38), and it has been suggested that ATP-phenylethanolamine complexes are somehow involved at the receptor sites (34). Spectroscopic studies have demonstrated the presence of phenylethanolamine ATP complexes in aqueous solution

(22,38) and one infra-red absorption study indicates that similar complexes occur *in vivo* in adrenal medullary storage granules (22).

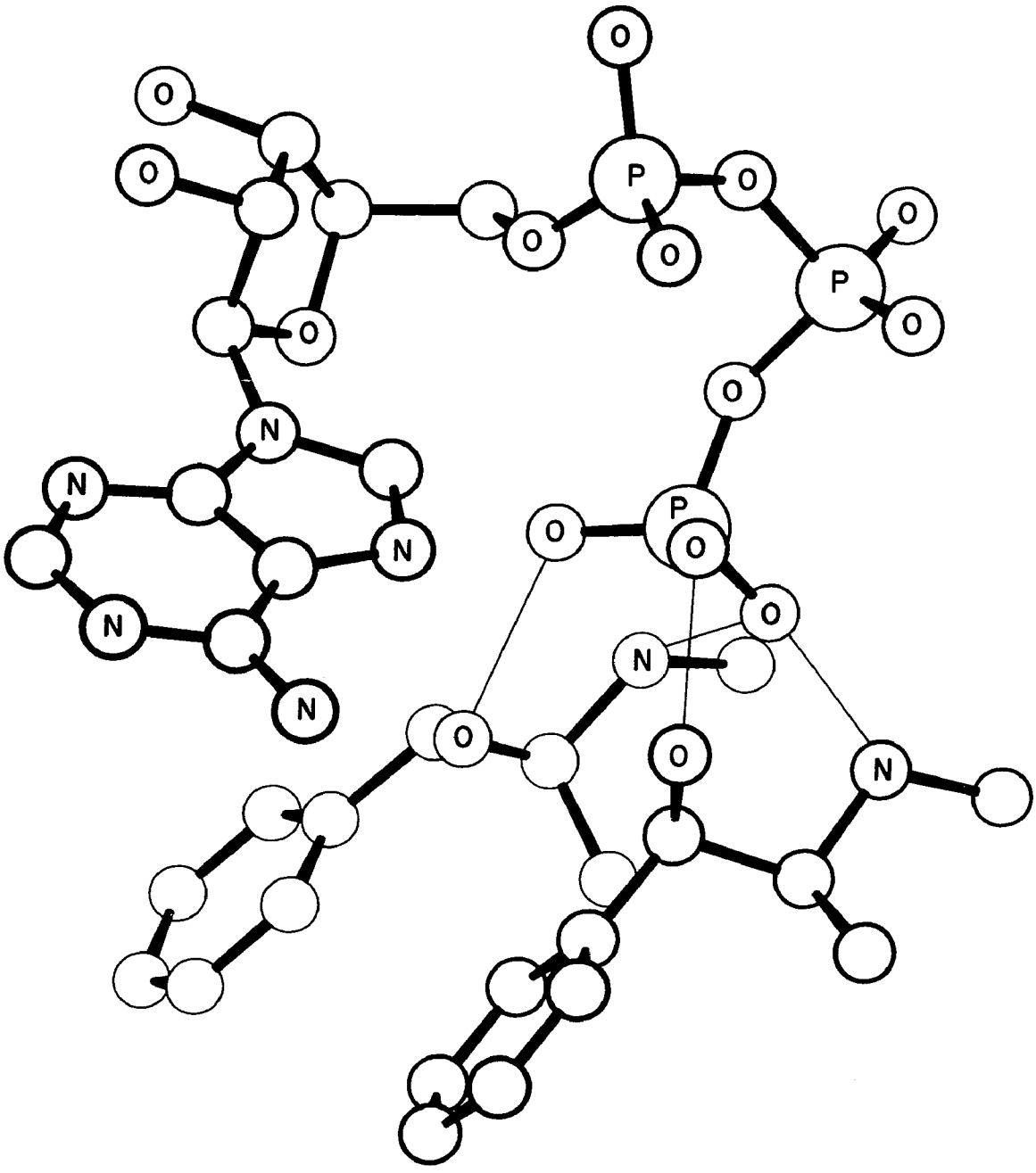
Three very important conclusions can be drawn from nuclear magnetic resonance (NMR) studies (38) of aqueous solutions consisting of a mixture of epinephrine and ATP. First, stacking interactions between the adenine ring and the aromatic ring of epinephrine apparently contribute little to the formation of the epinephrine ATP complex; second, since epinephrine binds equally well to ATP and AMP, it is likely that only one phosphate of ATP is involved; and third the hydroxyl group of the epinephrine is required for the binding of ATP, thus indicating that the interaction is not primarily due to coulombic attraction between the protonated amino group and the negatively charged phosphate moiety of ATP. This evidence suggests that, since the complex primarily involves binding between the ethanolamine moiety of epinephrine and a PO_4 group of ATP, the crystal structure of phosphate-ethanolamine complexes should serve as suitable model systems for understanding the mechanisms by which epinephrine and norepinephrine bind to ATP. Since the conformations of ephedrine and β-ethanolamine are closely related to those of epinephrine and norepinephrine, the same type of phosphate-ethanolamine hydrogen bonding occurring in the crystal structures of ephedrine monohydrogen phosphate and β-ethanolamine

monohydrogen phosphate should also govern phosphate interactions with the adrenergic phenylethanolamines.

Considering the conformational and phosphate-binding properties of phenylethanolamines, a model that is consistent with my crystallographic data can be composed of phenylethanolamine-ATP complexes. This model is shown in Figure 13, prepared by combining the actual structure of ATP (as determined from a crystallographic study of sodium ATP (15)) with the ephedrine-phosphate complex that I observed in the crystal structure of ephedrine monohydrogen phosphate. This combination was accomplished by replacing the terminal phosphate group of ATP with the HPO_4 anion from the ephedrine salt. When the phosphate group is transposed, along with the two ephedrine molecules, the resultant configuration is the one depicted in Figure 13. Because this configuration is derived directly from the precise results determined from the solid-state structures of ATP and ephedrine monohydrogen phosphate, and because it is consistent with the spectroscopic data on the phenylethanolamine-ATP complexes, it serves as a convincing model for the complexes that phenylethanolamines form with ATP in biological systems.

Figure 13.

Hypothetical ATP-ephedrine complex drawn by using the atomic coordinates of ATP and ephedrine from the crystal structure of sodium ATP and ephedrine phosphate. This combination was accomplished by replacing the terminal phosphate group of ATP with the HPO_4 anion from the ephedrine salt.


O
O
O
P
O
O
P
O
O
O
O
O
N
N
N
N
N
P
O
O
O
N
O
O
N

CONCLUSIONS

The results of those crystallographic studies, combined with previously existing information pertinent to β-ethanolamine phosphate interactions, lead to four major conclusions:

(1) β-ethanolamines generally assume a preferred conformation in which the α-hydroxyl group is situated gauche to the amino group. Although I believe this conformation is favored primarily because of a coulombic attraction between the amino cation and the electron rich hydroxyl group, it is likely that steric repulsion between the amino moiety and bulky substituents on C(7), such as the phenyl ring of phenylethanolamines, is also important in maintaining this preferred conformation.

(2) This preferred gauche conformation of β-ethanolamines established a specific geometrical arrangement of hydrogen bond donor sites (the amino and hydroxyl groups), thus limiting the geometries of hydrogen-bond acceptors that can interact with ethanolamines. The adjacent oxygen atoms in the phosphates have the proper spacing and geometry to serve as particularly suitable hydrogen-bond acceptor sites, and our crystallographic results demonstrate that any β-ethanolamine in this preferred conformation is

capable of forming a strong hydrogen-bonded complex with phosphate anions.

(3) Hydrogen bonding between phosphate anions and the β-ethanolamine moieties of hydroxylysine residues in bone collagen, may provide an important mechanism for the binding of phosphate minerals to the collagen matrices of bones and teeth. The sidechain of hydroxylysine would be expected to interact with phosphates through one of the two mechanisms suggested by these crystallographic studies (i.e. by interacting with dihydrogen phosphate arrays, or by binding single monohydrogen phosphate anions).

(4) This same type of phosphate β-ethanolamine interaction can account for the binding of epinephrine and norepinephrine to the phosphate moiety of ATP. It is important to emphasize that these crystallographic studies yield a type of interaction consistent with all available spectroscopic data. The formation of two hydrogen bonds from the phenylethanolamine to the phosphate anions of ATP is probably instrumental in establishing the ATP-phenylethanolamine complexes observed in adrenergic storage vesicles.

SUGGESTIONS FOR FUTURE RESEARCH

A number of questions concerning the conformational and phosphate-binding properties of ethanolamines remain unanswered, and require additional investigation. Many of these questions could be subjected to further study by X-ray diffraction and by spectroscopic methods, either of which might clarify the general biological importance of the interactions that have been observed in these crystallographic studies. Examples of the type of studies that are feasible and would be particularly useful include:

(1) Examination of the effects that divalent cations exert on the hydrogen-bonding between phosphates and ethanolamines. These crystallographic results have included rather idealized model systems, which contained only ethanolamines, phosphates, and water. However, for many biological processes, particularly mineralization processes, one would expect that calcium and magnesium ions might also be involved. Would these ions disrupt the phosphate-ethanolamine and phosphate-phosphate interactions that have been observed in this model system? This question could be answered by crystallizing ethanolamines as either calcium phosphate or magnesium phosphate complexes. Useful information concerning the effects of cations on the

solution interactions could be obtained by repeating the spectroscopic studies with calcium and magnesium ions included in the system.

(2) These crystallographic results have suggested a feasible model for ATP-phenylethanolamine complexes, and, although this model is compatible with spectroscopic data, it must be regarded as somewhat speculative until more direct structural data are obtained. Direct verification should be made possible by determining the crystal structure of a phenylethanolamine salt of ATP.

(3) The spectroscopic studies of phosphate-ethanolamine complexes have included only phenylethanolamines. My crystallographic results provide strong evidence that the same types of interactions should be possible for alkylethanolamines (e.g. the sidechain of hydroxylysine), and such evidence could be verified by spectroscopic investigations of aqueous solutions. The experimental approach established by the earlier spectroscopic studies of phenylethanolamine-ATP complexes, could be easily extended to include alkylethanolamine phosphates.

REFERENCES

1. Aoki, K., Kozo, N., and Iitaka, Y. The Crystal Structure of L-Arginine Phosphate Monohydrate. Acta Cryst. B27, 11-23 (1971).

2. Axelrod, J. and Weinshilboum, R. Catecholamines. Phys. in Med. 287, 237-242 (1972).

3. Bergin, R. Refinement of the Structure of (-)-Ephedrine Hydrochloride. Acta Cryst. B27, 381-386 (1971).

4. Busing, W. R., Martin, K. O., and Levy, H. A. ORXFLS, A Fortran Least-Squares Program. Report ORNL-TM-305, Oak Ridge National Laboratory, Tennessee (1962).

5. Carlstrom, D. and Bergin, R. The Structure of the Catecholamines. I. The Crystal Structure of Noradrenaline Hydrochloride. Acta Cryst. 23, 313-317 (1967).

6. Coppens, P. and Hamilton, W. C. Anisotropic Extinction Corrections in the Zachariasen Approximation. Acta Cryst. A26, 71-83 (1970).

7. Cromer, D. T. and Liberman, D. Relativistic Calculation of Anomalous Scattering Factors for X-Rays. J. Chem. Phys. 53, 1891-1898 (1970).

8. Dangoumau, J., Barrans, Y., and Cotrait, M. Conformations Moleculaires de Substances Adrenolytiques. J. Pharmacol. Paris 4 (1), 5-18 (1973).

9. Glimcher, M. J., Francois, C. J., Richards, L., and Krane, S. M. The Presence of Organic Phosphorous in Collagen and Gelatins. Biochim. Biophys. Acta 93, 585-602 (1964).

10. Glimcher, M. J. and Krane, M. The Incorporation of Radioactive Inorganic Orthophosphate as Organic Phosphate by Collagen Fibrils in vitro. Biochemistry 3, 195-202 (1964).

11. Huse, Y. and Iitaka, Y. The Crystal Structure of Spermine Phopshate Trihydrate. Acta Cryst. B25, 498-509 (1969).

12. Iitaka, Y. and Huse, Y. The Crystal Structure of Spermine Phosphate Hexahydrate. Acta Cryst. B18, 110-120 (1971).

13. Iversen, L. L. The Uptake and Storage of Noradrenaline in Sympathetic Nerves. New York: Cambridge University Press: New York (1967).

14. Johnson, C. K. ORTEP, A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations. Report ORNL-3794, revised, Oak Ridge National Laboratory, Tennessee (1965).

15. Kennard, O., Isaacs, N. W., Motherwell, W. D. S., Coppola, J. C., Wampler, D. L., Larson, A. C., and Watson, D. G. The Crystal and Molecular Structure of Adenosine Triphosphate. Proc. Roy. Soc. Lond. A. 325, 401-436 (1971).

16. Kier, L. B. The Preferred Conformations of Ephedrine Isomers and the Nature of the Alpha Adrenergic Receptor. J. Pharmacol. Exp. Ther. 164, 75-81 (1968).

17. Klyne, W. and Prelog, V. Description of Steric Relationships Across Single Bonds. Experientia 16, 521-523 (1960).

18. Kraut, J. The Crystal Structure of 2-Amino-Ethanol Phosphate. Acta Cryst. 14, 1146-1152 (1961).

19. Main, P., Woolfson, M. M., and Germain, G. MULTAN, A Computer Program for the Automatic Solution of Crystal Structures, Department of Physics, University of York, England, and Lab. de Chimie-Physique, Univ. de Louvain, Belgium (1971).

20. Mathew, M. and Palenik, G. J. The Crystal Structure of d,ℓ-Isoproterenol Sulfate Dihydrate. J. Amer. Chem. Soc. 93, 497-502 (1971).

21. Maynert, E. W., Moon, B. H., and Pai, V. S. Insoluble Solid Complexes of Norepinephrine and Adenosine Triphosphate. Mol. Pharmacol. 8, 88-94 (1972).

22. Pai, V. S. and Maynert, E. W. Interactions of Catecholamines with Adenosine Triphosphate in Solution and Adrenal Medullary Granules. Mol. Pharmacol. 8, 82-89 (1972).

23. Penn, R. E. and Curl, R. F. Microwave Spectrum of 2-Aminoethanol: Structural Effects of the Hydrogen Bond. J. Chem. Phys. 55, 651-658 (1971).

24. Philippot, E. and Lindqvist, O. The Crystal Structure of $KH_2(PO_4)_2$. Acta Chem. Scand. 25, 512-522 (1971).

25. Portoghese, P. S. Stereochemical Studies on Medicinal Agents. IV. Conformational Analysis of Ephedrine Isomers and Related Compounds. J. Med. Chem. 10, 1057-1063 (1967).

26. Preston, H. S. and Steward, J. M. The Crystal Structure of the Antimalarial Chloroquine Diphosphate Monohydrate. J. Chem. Soc. D 18, 1142-1143 (1970).

27. Putkey, E. and Sundaralingam, M. Molecular Structures of Amino Acids and Peptides. I. The Crystal Structure and Conformation of DL-O-Serine Phosphate Monohydrate. Very Short Phosphate-Phosphate Hydrogen Bonds. Acta Cryst. B26, 782-790 (1972).

28. Santanum, M. S. Calcification of Collagen. J. Mol. Biol. 1, 65-68 (1959).

29. Schiffman, E., Martin, G. R., and Miller, E. J., "Matrices that Calcify", In Biological Calcification: Cellular and Molecular Aspects. Ed. H. Schraer, New York: Meredith Corporation (1970).

30. Selvaratnam, M. and Spiro, M. The Transference Numbers of Orthophosphate Acid and the Limiting Equivalent Conductance of the H_2PO_4 Ion in Water at 25°C. Trans. Faraday Soc. 61, 360-373 (1965).

31. Shefter, E. In Cholinergic Ligand Interactions. Eds. D. J. Triggle, J. F. Moran, E. A. Barnad, New York: Academic Press, p. 83 (1971).

32. Solomons, C. C. and Irving, J. T. Studies in Calcification. The Reaction of Some Hard- and Soft-Tissue Collagen with 1-Fluoro-2:4-Dinitrobenzene. Biochem. J. 68, 499-503 (1958).

33. Stewart, R. F., Davidson, E. R., and Simpson, W. T. Coherent X-Ray Scattering for the Hydrogen Atom in the Hydrogen Molecule. J. Chem. Phys. 42, 3175-3187 (1965).

34. Sundaralingam, M. and Putkey, E. F. Molecular Structures of Amino Acids and Peptides. II. A Redetermination of the Crystal Structure of L-O-Serine Phosphate. A Very Short Phosphate-Carboxyl Hydrogen Bond. Acta Cryst. B26, 790-800 (1972).

35. Triggle, D. J. "Adrenergic Hormones and Drugs". In Medicinal Chemistry, 3rd Edition, Part II. Ed. A. Burger, New York: Wiley-Interscience (1970).

36. Vedeis, M. V., Palenik, E. F., Schaffrin, R. and Trotter, J. Crystal Structure of Histamine Diphosphate Monohydrate. J. Chem. Soc. A, 2659-2666 (1969).

37. Wehe, D. J., Busing, W. R., and Levy, H. A. ORABS, A Fortran Program for Calculating Single Crystal Absorption Corrections. Report ORNL-TM-229, Oak Ridge National Laboratory, Tennessee (1962).

38. Weimer, N. and Jardetzky, O. A Study of Catecholamine Nucleotide Complexes by Nuclear Magnetic Resonance Spectroscopy. Naunym-Schmiedebergs Arch. exp. Path. u. Pharmak. 248, 308-318 (1964).

39. Wilson, A. J. C. Determination of Absolute from Relative X-Ray Intensity Data. Nature (London) 150, 151-152 (1942).

40. Wuthier, R. E., Grøn, P., and Irving, J. T. The Reaction of 1-Fluoro-2,4-Dinitrobenzene with Bone. Biochem. J. 92, 205-216 (1964).

41. Zachariasen, W. H. The Secondary Extinction Correction. Acta Cryst. 16, 1139-1146 (1963).

42. International Tables for X-Ray Crystallography, Volume III, Birmingham: Kynoch Press, pp. 202-214 (1962).

GRADUATE SCHOOL
UNIVERSITY OF ALABAMA IN BIRMINGHAM
DISSERTATION APPROVAL FORM

Name of Candidate Richard A. Hearn

Major Subject Biochemistry

Title of Dissertation Crystallographic Studies of the Conformational and Phosphate Binding Properties of Ethanolamines.

Dissertation Committee:

Charles E. Bugg, Chairman

Robert B. Sutton

William J. Butler

Hubert Chang

James C. Lacey Jr.

Jean M. Olson

Director of Graduate Program John M. McKelvey

Dean, UAB Graduate School S. B. Barker by W. B. Harper

Date 7 June '74