Crystal and Molecular Structure of 8-Azaestradiol

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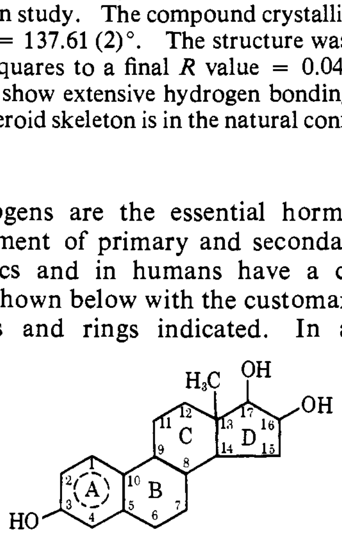
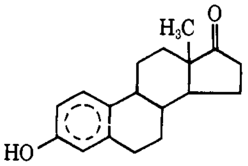
Abstract: The crystal and molecular structure of 8-azaestradiol has been determined by a single crystal X-ray diffraction study. The compound crystallizes in space group $P2_1/c$ with $a = 9.782$ (3), $b = 19.957$ (1), $c = 10.947$ (4) Å, and $\beta = 137.61$ (2)°. The structure was solved by the symbolic addition procedure and refined by block diagonal least squares to a final R value $= 0.04$ for the 1373 statistically significant reflections. The hydroxyl hydrogen atoms show extensive hydrogen bonding to the nitrogen and oxygen with distances 1.92 and 1.78 Å, respectively. The steroid skeleton is in the natural configuration with intramolecular parameters similar to those of estradiol.

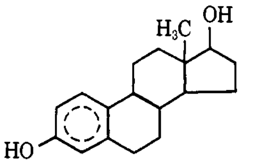
The estrogens are the essential hormones for the development of primary and secondary female sex characteristics and in humans have a common ring skeleton as shown below with the customary numbering of positions and rings indicated. In addition, the natural configuration has α hydrogens at the 9 and 14 positions and a β hydrogen at the 8 position (the methyl group at the 13 position defines the β orientation).



(I)
estriol



(II)
estrone



(III)
estradiol

To avoid unwanted pharmacological side-effects and in hopes of enhancing desirable medicinal properties,[1] aza analogs of the steroids have been synthesized[2,3] and for the 8-aza series, the structural and conformational assignments have recently been characterized.[4,5] For 8-azaestrone[6] the intramolecular conformation and parameters were found to be identical with those of the 4-bromoestrone[7] and therefore its lack of estrogenic activity could not be attributed to any large conformational changes nor to any variation from the "idealized intramolecular O····O distance" supposedly characteristic of estrogens,[8] but rather to the lability of the hydroxyl hydrogen at the 3 position. In the crystal structures of 8-azaestrone hydrobromide[6] and 12-keto-17-deoxo-8-azaestrone hydrobromide,[4] the bromide ion disrupted the pattern of hydrogen bonding observed in 4-bromoestrone,[7] estriol,[9] and 4-bromoestradiol.[10] In addition, the C–N distances about the quaternary aza analogs showed no significant differences from natural estrones. It thus became imperative to investigate one of the aza analogs as the free base (with a tertiary rather than a quaternary nitrogen). Consequently, the structure of 8-azaestradiol was undertaken.

Experimental Section

Barrel shaped crystals of 8-azaestradiol,[11] $C_{17}NO_2H_{23}$, were grown by slow evaporation of a solution in 1-propanol.[12] The symmetry and cell data were determined from a single crystal measuring 0.09 × 0.24 mm on a General Electric XRD-5 diffractometer using Cu Kα radiation. Lattice constants were determined by a least-squares fit of 16 carefully measured 2θ values (1° take-off angle and 0.05° slit) at $2\theta > 68°$ where the Cu Kα_1 and Kα_2 doublet is resolvable.

Three-dimensional intensity data were collected on a General Electric XRD-490 fully automated diffractometer by the stationary-counter, stationary-crystal method using balanced nickel and cobalt filters and Cu Kα radiation. A total of 2135 independent reflections were measured to a 2θ maximum of 130° ($d = 0.85$ Å). Of these, 1373 reflections (64%) were considered statistically acceptable when $(I_{\mathrm{Ni}} - 2\sigma(I_{\mathrm{Ni}})) - (I_{\mathrm{Co}} + 2\sigma(I_{\mathrm{Co}})) > 125$ counts, 10 sec counting time. The data were corrected for α_1-α_2 splitting[13] as a function of 2θ and for absorption as a function of ϕ (no more than 4% difference in a ϕ scan at $\chi = 90°$). Lorentz polarization correc-

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[1] M. Martin-Smith and M. G. Sugane, *J. Pharm. Pharmacol.*, **16**, 569 (1964).

[2] R. E. Brown, D. M. Lustgarten, R. J. Stanaback, and R. I. Meltzer, *J. Org. Chem.*, **31**, 1489 (1966).

[3] A. H. Reine and A. I. Meyers, *ibid.*, **35**, 554 (1970).

[4] J. N. Brown, R. L. R. Towns, and L. M. Trefonas, *J. Heterocycl. Chem.*, **8**, 273 (1971).

[5] R. E. Brown, A. I. Meyers, L. M. Trefonas, R. L. R. Towns, and J. N. Brown, *ibid.*, **8**, 279 (1971).

[6] R. Majeste and L. M. Trefonas, *J. Amer. Chem. Soc.*, **91**, 1508 (1969).

[7] D. A. Norton, G. Kartha, and C. T. Lu, *Acta Crystallogr.*, **16**, 89 (1963).

[8] M. Oki, *J. Chem. Soc. Jap.*, 73, 252 (1952).

[9] A. Cooper, D. A. Norton, and H. Hauptman, *Acta Crystallogr., Sect. B*, **25**, 814 (1969).

[10] D. A. Wortory, G. Kartha, and C. T. Lu, *Acta Crystallogr.*, **17**, 77 (1964).

[11] 8-Azaestradiol was provided by the Warner-Lambert Research Institute, Morris Plains, N. J., research affiliate of Warner-Chilcott Laboratories.

[12] Rectangularly shaped crystals grown from ethanol were found to be in space group $P222_1$, with $a = 9.47$, $b = 7.97$, and $c = 22.47$ Å. Interestingly enough, these cell dimensions would lead to a density of 1.07 g/cm³ assuming four molecules per cell compared to the calculated density of 1.26 g/cm³ in $P2_1/c$. Furthermore, since this is a noncentrosymmetric space group, whereas $P2_1/c$ is centrosymmetric, this would imply a resolution of the optically active form by change of solvent. Both facts can be reconciled by one assumption. When the more polar solvent is used (ethanol), hydrogen bonding with the solvent is more likely, leading to a crystal form which contains bound solvent and which would continually utilize whichever optically active form was involved in the initial crystallization. Thus, the crystals obtained from ethanol should be in the noncentrosymmetric space group (they are, namely $P222_1$) and the calculated density in this case should be recalculated to include at least one ethanol molecule (this would give a calculated density of 1.25 g/cm³, comparable to the 1.26 g/cm³ obtained from propanol $P2_1/c$).

[13] A. Tulinsky, C. Worthington, and E. Pignataro, *Acta Crystallogr.*, **12**, 623 (1959).

Table I. Fractional Coordinates and Anisotropic Thermal Parameters[a]
(Anisotropic Thermal Parameters $\times 10^4$, Estimated Standard Deviations in Parentheses Refer to Last Decimal Place)

Atom	X	Y	Z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C-1	0.4602 (4)	0.5086 (1)	0.6829 (3)	134 (9)	16 (1)	94 (6)	−12 (2)	62 (7)	−2 (2)
C-2	0.4991 (4)	0.5634 (1)	0.6343 (4)	147 (9)	14 (1)	121 (7)	−9 (2)	81 (7)	−1 (2)
C-3	0.3530 (4)	0.5847 (1)	0.4560 (4)	162 (9)	13 (1)	150 (7)	0 (2)	121 (7)	7 (2)
C-4	0.1719 (4)	0.5497 (1)	0.3275 (3)	145 (9)	15 (1)	114 (6)	1 (2)	87 (7)	6 (2)
C-5	0.1340 (4)	0.4940 (1)	0.3761 (3)	115 (8)	13 (1)	95 (6)	5 (2)	76 (6)	0 (2)
C-6	−0.0596 (4)	0.4552 (1)	0.2324 (3)	132 (9)	15 (1)	81 (6)	0 (2)	57 (6)	1 (2)
C-7	−0.1032 (4)	0.4069 (1)	0.3077 (3)	98 (8)	15 (1)	87 (6)	2 (2)	46 (6)	4 (2)
N-8	0.0807 (3)	0.3693 (1)	0.4646 (3)	85 (6)	11 (1)	68 (4)	0 (2)	53 (5)	1 (1)
C-9	0.2297 (4)	0.4177 (1)	0.6162 (3)	124 (8)	13 (1)	79 (6)	−6 (2)	67 (6)	−4 (2)
C-10	0.2775 (4)	0.4735 (1)	0.5571 (3)	116 (8)	10 (1)	98 (6)	0 (2)	79 (6)	−1 (2)
C-11	0.4173 (4)	0.3818 (1)	0.7885 (3)	136 (9)	20 (1)	72 (6)	−14 (2)	31 (6)	7 (2)
C-12	0.3654 (4)	0.3276 (1)	0.8482 (3)	143 (9)	20 (1)	78 (6)	−10 (2)	57 (6)	3 (2)
C-13	0.2091 (4)	0.2800 (1)	0.6921 (3)	106 (8)	13 (1)	85 (6)	−1 (2)	72 (6)	2 (2)
C-14	0.0278 (4)	0.3217 (1)	0.5278 (3)	106 (8)	15 (1)	86 (6)	−3 (2)	75 (6)	−3 (2)
C-15	−0.1379 (4)	0.2698 (1)	0.3930 (3)	115 (8)	18 (1)	89 (6)	−12 (2)	53 (6)	−1 (2)
C-16	−0.0971 (4)	0.2153 (1)	0.5164 (4)	173 (9)	24 (1)	122 (7)	−17 (3)	93 (7)	2 (2)
C-17	0.1000 (4)	0.2355 (1)	0.7114 (3)	130 (8)	14 (1)	100 (6)	0 (2)	89 (6)	5 (2)
M-13	0.3035 (4)	0.2366 (1)	0.6544 (4)	193 (10)	21 (1)	166 (7)	22 (3)	157 (7)	23 (2)
O-3	0.3796 (3)	0.6394 (1)	0.4004 (2)	218 (6)	19 (1)	177 (5)	−13 (2)	125 (5)	15 (1)
O-17	0.2199 (3)	0.1800 (1)	0.8299 (2)	158 (6)	16 (1)	128 (4)	11 (2)	116 (4)	16 (1)

[a] Anisotropic temperature factors of the form $\exp -[\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl]$.

Table II. Hydrogen Fractional Coordinates
(Isotropic Thermal Parameter Fixed at 4.0 Å^2)

Atom[a]	X	Y	Z
H1	0.5677 (32)	0.4962 (11)	0.8186 (29)
H2	0.6358 (34)	0.5857 (11)	0.7310 (30)
H4	0.0602 (34)	0.5626 (11)	0.1882 (29)
H6	−0.0595 (33)	0.4314 (11)	0.1500 (29)
H6′	−0.1816 (33)	0.4878 (11)	0.1393 (29)
H7	−0.2133 (34)	0.3749 (11)	0.2059 (30)
H7′	−0.1657 (33)	0.4299 (11)	0.3382 (29)
H9	0.1598 (33)	0.4416 (11)	0.6440 (29)
H11	0.5085 (33)	0.4171 (11)	0.8918 (29)
H11′	0.5007 (34)	0.3647 (11)	0.7710 (31)
H12	0.2976 (32)	0.3515 (11)	0.8839 (29)
H12′	0.4861 (35)	0.3051 (11)	0.9570 (30)
H14	−0.0180 (33)	0.3473 (11)	0.5749 (29)
H15	−0.1348 (34)	0.2507 (11)	0.3079 (29)
H15′	−0.2763 (34)	0.2905 (10)	0.3077 (30)
H16	−0.0809 (33)	0.1690 (11)	0.4835 (30)
H16′	−0.2072 (34)	0.2107 (11)	0.5036 (30)
H17	0.0730 (34)	0.2642 (11)	0.7724 (31)
HM13	0.3497 (34)	0.2629 (11)	0.6155 (29)
HM13′	0.1933 (34)	0.2010 (11)	0.5448 (30)
HM13″	0.4195 (34)	0.2107 (11)	0.7647 (30)
HO3	0.5164 (35)	0.6571 (11)	0.4974 (31)
HO17	0.1816 (33)	0.1638 (11)	0.8780 (29)

[a] Numbers following H refer to bonding atom.

tions were made and the intensities were reduced to structure amplitudes in the usual manner. Crystal data for 8-azaestradiol, $C_{17}H_{23}NO_2$, $M = 274$, are: monoclinic, $a = 9.782 \pm 0.003$, $b = 19.957 \pm 0.001$, $c = 10.947 \pm 0.004$ Å; $\beta = 137.61 \pm 0.02°$; $F(000) = 596$; for $z = 4$, $d_c = 1.26$; space group $P2_1/c$ (C_{2h}^5) from systematic absences; linear absorption coefficient, μ (Cu Kα) = 6.55 cm^{-1}.

Structure Determination

Normalized structure magnitudes, $|E|$'s, were calculated using a K curve[14] and the phases of 213 E's (from a total of 232 with $|E| > 1.5$) were determined by the symbolic addition procedure[15] in terms of one symbolic sign, no phase being accepted below a probability of 0.999. The entire steroid skeleton was present in one of the two E maps whereas the other contained only random peaks. Several cycles of block diagonal least-squares refinement of the coordinates and isotropic temperature factors for the 20 nonhydrogen atoms using $1/\sigma^2$ weights led to a value of $R = 0.20$. This was followed by conversion of the isotropic temperature factors to anisotropic temperature factors and several more cycles of similar refinement which gave an R value = 0.13.

The hydrogen atoms were located from a difference electron density map phased by the nonhydrogen atoms. The refinement was continued varying the anisotropic temperature factors of the nonhydrogen atoms and the coordinates of all atoms. The temperature factors of the hydrogen atoms were held fixed at 4.0 Å^2. The refinement converged at a value of $R = 0.04$ with all shifts less than one-tenth the estimated standard deviation of the respective parameter.

Discussion

Table I lists the final coordinates and anisotropic temperature factors for all nonhydrogen atoms and Table II lists the final coordinates of the hydrogen atoms. The estimated standard deviations (esd) in the bond distances and angles, as calculated from the least-squares matrix, are less than 0.005 Å and 0.3°, respectively, for nonhydrogen atoms. Figure 1 shows the distances and angles within the steroid skeleton. Figure 2 is a projection of the contents of the unit cell along (00l) indicating the intermolecular hydrogen bonding scheme. Finally, Figure 3 is an ORTEP[16] stereo drawing of the molecule.[17]

The steroid is in the natural configuration with hydrogens at the 9 and 14 positions assigned as α with respect to the methyl group at the 13 position which, by convention, is designated as β. Ring A (the phenyl ring) was fitted to the "best" plane by least-squares methods and conformed to this plane with an esd value of 0.01 Å. Rings B and C are in the chair conformation.

In general, the molecular parameters are in agreement with those reported for the aforementioned steroid

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(14) J. Karle, H. Hauptman, and C. L. Christ, *Acta Crystallogr.*, **11**, 757 (1958).

(15) The symbolic addition procedure was computed *via* the program DIRECT written in Fortran IV by J. N. Brown for the PDP-10 computer (1969).

(16) C. K. Johnson, ORTEP, ORNL-3794, Oak Ridge National Laboratories, Nashville, Tenn. (1965).

(17) Additional structure factor tables will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-72-4311. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

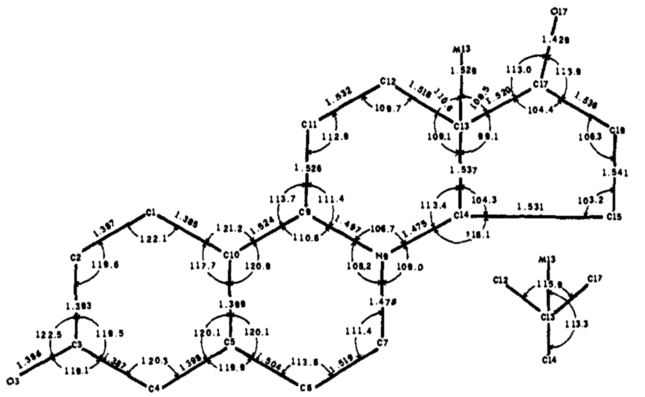

Figure 1. Estradiol skeleton with distances and angles indicated.

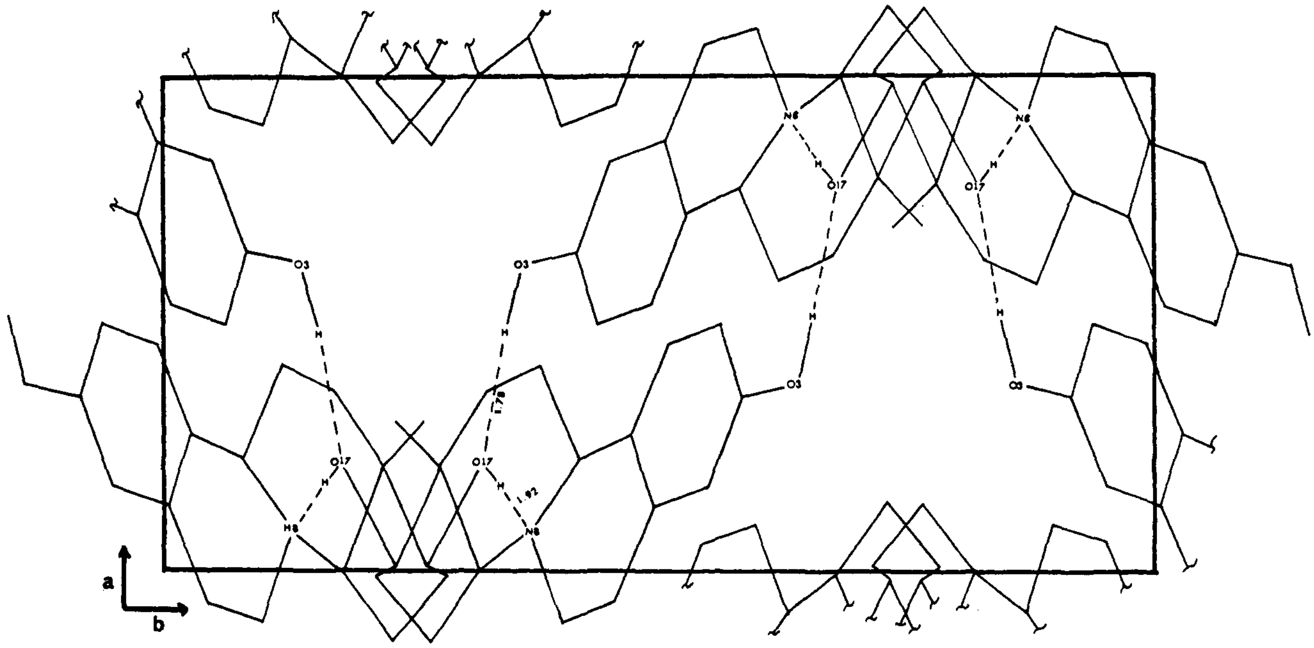

Figure 2. Projection of contents of unit cell down the *c* axis. Intermolecular hydrogen bonding scheme indicated.

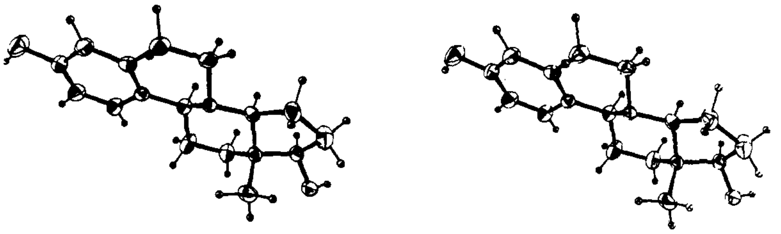

Figure 3. ORTEP stereodrawing of the molecule.

structures to within two esd's except at the diol positions and at the aza position. All C–C distances within the aromatic ring A are within two esd's of the accepted C–C distance in benzene, 1.396 Å, except for the shortened C-2–C-3 distance, 1.383 Å, which is intermediate between the corresponding distance in estriol, 1.377 Å, and that in benzene. The enlarged angle C-2–C-1–C-10, 122.1°, and the small angle C-1–C-10–C-5, 117.7°, parallel the results reported in the previous steroid studies.

Rings B and C differ from the other 8-azasteroid studies at the 8 position. This structure shows for the first time the shortening of the C–N distances about the tertiary nitrogen (N-8–C-7, 1.479 Å; N-8–C-9, 1.497 Å; N-8–C-14, 1.475 Å) as opposed to those about the quaternary nitrogen in 8-azaestrone hydrobromide (1.51, 1.54, 1.53 Å, respectively) and in 12-keto-17-deoxo-8-azaestrone methyl ether hydrobromide (1.510, 1.526, 1.522 Å, respectively). The distances and angles within ring D are in agreement with estradiol and estriol including the seemingly small C-14–C-13–C-17 angle, 99.1°.

The C–O distances at the 3 position, 1.366 Å, and that at the 17 position, 1.428 Å, differ by 15 esd's although both are consistent with the identical parameters reported in the estriol structure study.[9] The distance 1.36 Å is observed in phenolic groups[18,19] and suggests that the shortening of the C–O bond at the 3 position is due to delocalization into the aromatic system of ring A. Distances of 1.43 Å are observed in aliphatic alcohols[20] and are in agreement with the C–O bond at the 17 position. The hydroxyl group bond distance, O-3–HO-3, 0.97 ± 0.03 Å, is longer than the corresponding O-17–HO-17 bond distance, 0.90 ± 0.03 Å,

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(18) P. Coppens and G. M. J. Schmidt, *Acta Crystallogr.*, **18**, 62 (1965).

(19) P. Coppens and G. M. J. Schmidt, *ibid.*, **18**, 654 (1965).

(20) D. C. Fries, S. T. Rao, and M. Sundaralingam, *Acta Crystallogr., Sect. B*, **27**, 994 (1971).

by slightly more than two esd's, suggesting that the delocalization of the C–O bond at the 3 position has weakened the O–H bond there, thus making the 3 position hydroxyl hydrogen more labile. 8-Azaestradiol has only weak estrogenic activity. A private communication from R. E. Brown of Warner-Lambert Pharmaceuticals (Sept. 27, 1971) states... "Uterotrophic activity was assayed by the procedure of Rubin, *et al.* [*Endocrinology*, **49** (1951)] by Dr. R. Kroc and Mr. C. Rassaert of the Warner-Lambert Research Institute. At a dose of 150 mg/kg po, 8-azaestradiol was practically devoid (0.01% that of ethynyl estradiol) of estrogenic activity...." Yet this compound shows almost identical molecular parameters with estradiol. Therefore, its lack of estrogenic activity must be caused by the competition between the admittedly weak protonation at the phenolic oxygen (attached to position 3) and the potential protonation of the nitrogen at the 8 position during the initial glucuronidation. Without this acid-catalyzed protonation at the normal 3 position, the sequence of reactions necessary to stimulate estrogenic activity will be suppressed.

The two previous studies of azasteroids showed no hydrogen bonding between either the oxygen or nitrogen. This was attributed to the presence of the bromide ion. This study does show hydrogen bonding similar to that in estriol. The hydrogen bonded to O-3 is 1.78 Å from O-17 (in equivalent position $1 - x$, $\frac{1}{2} + y$, $\frac{3}{2} - z$) and forms the angle O-3–H····O-17, 172°, whereas in estriol the corresponding parameters are 1.80 Å and 177°, respectively. In addition, the hydrogen at O-17 is 1.92 Å from the nitrogen (equivalent position x, $\frac{1}{2} - y$, $\frac{1}{2} + z$) and forms the angle O-17–H····N, 176°.

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Stereochemistry of Nucleic Acids and Their Constituents. XXVI. The Crystal and Molecular Structure of a 1 : 1 Adduct of Ethanol and 3′-Deoxy-3′-(dihydroxyphosphinylmethyl)adenosine, an Analog of Adenosine 3′-Monophosphate[1]

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Abstract: The structure of 3′-deoxy-3′-(dihydroxyphosphinylmethyl)adenosine a synthetic analog of adenosine 3′-phosphate, has been determined using 1417 reflections measured on a diffractometer. The compound crystallized in the space group $P2_12_12_1$ with four molecules of the analog and four molecules of ethanol in a unit cell having $a = 5.592$, $b = 20.287$, and $c = 15.198$ Å; $d_{\mathrm{calcd}} = 1.507$ g cm^{-3} and $d_{\mathrm{obsd}} = 1.503$ g cm^{-3}. The observed and calculated densities are consistent with the presence of four molecules of ethanol per unit cell, which was subsequently confirmed by X-ray analysis. The molecule is in the anti conformation with respect to the glycosyl torsion angle, having $\chi = 28.1°$. The conformation about the C(4′)–C(5′) bond is gauche–gauche, where O(1′)–C(4′)–C(5′)–O(5′) = −69.0° and C(3′)–C(4′)–C(5′)–O(5′) = 49.2°. The conformation of the sugar ring is $^3T_2$, the torsion angles about the ring bonds being O(1′)–C(1′) = 8.9°, C(1′)–C(2′) = −29.5°, C(2′)–C(3′) = 37.4°, C(3′)–C(4′) = −33.1°, C(4′)–O(1′) = 15.4°. These conformational parameters are similar to those found in adenosine 3′-phosphate dihydrate. The molecule is a zwitterion N(1) of the base being protonated by an adjacent phosphonate hydrogen. The same phosphonate group is also hydrogen bonded to N(6) of the base. Similarly, the second hydrogen on N(6) and the site N(7) are involved in a hydrogen-bonded pair to a symmetry related phosphonate group. The base–phosphonate (or phosphate) hydrogen bonding is a characteristic feature of the crystal chemistry of adenine and cytidine nucleotides. The alcohol of solvation is hydrogen bonded to an adjacent ribose O(5′) atom. The remaining potential hydrogen bonding sites are also involved in hydrogen bonding.

The syntheses of phosphonate analogs of nucleoside phosphates have been of considerable interest since the compounds are of use in the study of the mechanisms of enzyme and hormone action.[2] This, in turn, makes a description of the three-dimensional structures of these analogs, relative to the structures of the naturally occurring phosphates, of particular interest in terms of the physical basis of their biological activities. The structure of adenosine 3′-phosphate dihydrate (3′-AMP) has been described in detail previously.[3] We now describe the structure of 3′-deoxy-

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(1) For part XXV of this series, see P. Pyusiner and M. Sundaralingam, *Acta Crystallogr.*, in press.

(2) H. P. Albrecht, G. H. Jones, and J. G. Moffatt, *J. Amer. Chem. Soc.*, 92, 5511 (1970).

(3) M. Sundaralingam, *Acta Crystallogr.*, 21, 495 (1966).