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Refinement of the Crystal Structure of Adenosine-5'-Phosphate

BY J. KRAUT

Department of Biochemistry, University of Washington, Seattle, Washington, U.S.A.

AND L. H. JENSEN

Department of Anatomy, University of Washington, Seattle, Washington, U.S.A.

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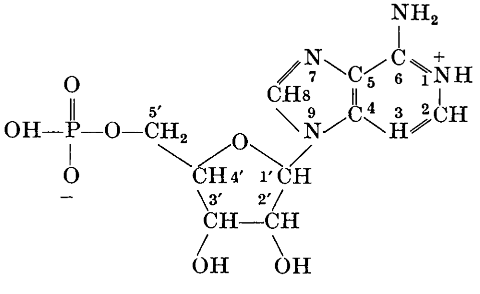
The crystal structure of adenosine-5'-phosphate has been determined by three-dimensional Patterson superposition and two-dimensional Fourier methods. It has been refined by full-matrix least-squares computations to a final R factor of 6·8% for 1197 reflections with measurable intensities. The space group is $P2_1$, with unit cell dimensions of $a = 12{\cdot}77$, $b = 11{\cdot}82$, $c = 4{\cdot}882$ Å and $\beta = 92° 24'$. The locations of 11 out of 16 hydrogen atoms in the asymmetric unit have been determined by difference-Fourier syntheses and least-squares refinement.

The adenine ring is essentially unchanged from adenine hydrochloride, though it is slightly non-planar, probably owing to packing forces. The N-glycosidic bond is bent about 8° out of the adenine plane. Atom C3' of the ribose ring is 0·66 Å out of the plane of the remaining four atoms. The relative orientation of the adenine and ribose groups is *anti*, with $\varphi_{CN} = -18°$. Certain generalities are suggested concerning the structure of organic phosphate groups.

Introduction

In addition to the fundamental role they play in the various nucleic acids, adenine nucleotides also serve a variety of specialized functions. It will be recalled, for example, that adenylic acid is a coenzyme in the activation of amino acids for protein synthesis; that adenosine triphosphate (*ATP*) is the apparently universal medium for transfer of biological energy; and that the adenylic acid group occurs in a variety of ubiquitous coenzymes: diphosphopyridine nucleotide (*DPN*), flavin-adenine-dinucleotide (*FAD*) and coenzyme *A*.

The conventional formula and numbering system for adenosine-5'-phosphate is:



The same compound is also variously referred to as adenylic acid, muscle adenylic acid and adenosine monophosphate (*AMP*). Its systematic name is 9-β-5'-phospho-D-ribofuranosyladenine. An arbitrary atom identification system will be used throughout this paper. It may be interpreted by reference to Fig. 1.

To date there has been reported only one precise three-dimensional structure determination of a nucleotide, that of calcium thymidylate by Trueblood, Horn & Luzzati (1961). Alver & Furberg (1959) have published the structure of cytidine-3'-phosphate based on refinement of two projections, and a single projection of adenosine-3'-phosphate has been published by Brown *et al.* (1953). A preliminary note on the crystal structure of adenylic acid has already appeared (Kraut & Jensen, 1960).

In the realm of the polynucleotides, the structure of *DNA* first proposed by Watson & Crick (1953) and Crick & Watson (1954) is now widely accepted. This has been further refined by Langridge *et al.* (1960*a*, *b*). *RNA* fibers on the other hand give much poorer X-ray diffraction pictures and the *RNA* structure is still unknown. That is to say, there has been no proposal of a structure with definite bond lengths and angles which may be compared with accepted values. Indeed it seems doubtful that *RNA* possesses a unique regular structure at all. Rich *et al.* (1961) have, however, assigned a definite structure to polyadenylic acid, the parameters of which are compared with the molecular parameters of adenylic acid in a subsequent section of this paper.

Experimental

Commercial adenosine-5'-phosphate (Nutritional Biochemicals Co.) was crystallized as long thin needles by slow evaporation of an aqueous solution adjusted to pH 2–3 with a trace of HCl. These were observed to have monoclinic symmetry. Unit-cell parameters were measured on Weissenberg films obtained with Cu $K\alpha$ radiation, using a camera calibrated against NaCl, $a = 5{\cdot}6394$ Å assumed. A wavelength of 1·5418 Å was assumed for the mean of $K\alpha_1$ and $K\alpha_2$. The unit-cell parameters were found to be

$$a=12\cdot77\pm0\cdot02,\ b=11\cdot82\pm0\cdot02,\ c=4\cdot882\pm0\cdot01\ \text{Å};\ \beta=92°\ 24'\pm5'.$$

The uncertainties indicated are estimated standard deviations. The needle axis of the crystal habit was parallel to the unit-cell c axis.

Although experimental density data are not available (owing to our inability to reproduce this particular crystal form at a later date), a reasonable calculated density of 1·647 g.cm.$^{-3}$ is obtained on the assumption of two molecules of the monohydrate per unit cell. Systematic absence of $0k0$ reflections with k odd indicated $P2_1$ or $P2_1/m$ as the possible space groups, and $P2_1$ was chosen as probably correct in view of the reasonableness of the calculated density and the likelihood that the unit cell contains two optically active molecules.

Unidimensionally integrated Weissenberg photographs of levels of constant l, out to $l=4$, and of the level $h0l$ were taken with Cu $K\alpha$ radiation. The crystals used were small, measuring a few hundredths of a millimeter perpendicular to the needle axis and about 0·5 mm. long. Densitometer tracings perpendicular to the direction of photographic integration were planimetered for each reflection to give integrated intensity data. Because of the small size of the crystals long exposures were necessary, and the resulting high film background somewhat impaired the accuracy of the photometric measurements. The various l-levels were placed on a common scale by means of the $h0l$ data. No correction was made for absorption effects.

Of the total of approximately 1700 unique reflections within the Cu $K\alpha$ limit, intensity data were obtained for 1487, or 87%. Of these 290, or 19%, were too weak to measure.

Determination of the structure

It will be appreciated that some interesting obstacles to the solution of this crystal structure are present. A long b-axis makes it unlikely that the heavy-atom method can be applied to the centrosymmetric projection. In fact this approach was tried very early without success. In the short c-projection, on the other hand, as well as in three dimensions, the heavy-atom based Fourier synthesis would contain a spurious mirror plane. This complicating feature, added to the fact that a phosphorus atom is probably not sufficiently heavy to give good phase determination by itself, made this approach seem unpromising.

The difficulty was overcome by the application of superposition methods to the sharpened Patterson function. In essence, the solution was made possible by the remarkable clarity with which the individual atoms of a structure stand out in sharpened Patterson superpositions. Thus, although the mirror-image ambiguity remains, exactly as in Fourier methods, one is nevertheless able to separate the two images without great difficulty. In the following paragraphs we outline the procedure that was actually followed.

A sharpened, origin-removed three-dimensional Patterson synthesis was computed and the approximate x and z coordinates of the phosphorus atom determined by assuming that the highest peak of the Harker section represented the phosphorus–phosphorus interaction. A twofold minimum-function superposition was computed on the basis of these phosphorus coordinates, resulting in a function with the symmetry of space group $P2_1/m$. Theoretically this map contained the desired structure, but accompanied as well by a reflection of the molecule in the set of mirror planes perpendicular to the b-axis. The mirror planes, in fact, must pass through each of two symmetry-related phosphorus atoms.

A three-dimensional model of the superposition map was next constructed, in which each of the 24 highest peaks in the asymmetric unit was simply represented by a clay ball. The phosphate group, practically coincident with its own mirror image, and much of the adenine ring were immediately obvious. The rest of the molecule was eventually found by working back and forth between successive Fourier c-projections and the model of the superposition map.

When the complete structure was assembled, it was realized in retrospect that the molecule could have been picked out entirely from the superposition map model without recourse to Fourier methods. However, since this was one of our earliest experiments with the technique, we probably lacked sufficient confidence in its power to make the necessary effort.

Refinement

The following atomic scattering factors were used in the refinement: hydrogen, carbon, nitrogen and oxygen of McWeeny (1951); phosphorus from *Internationale Tabellen* (1935).

The first stage of refinement was carried out by means of difference-Fourier syntheses in the b- and c-projections. Coordinates were adjusted, maintaining a constant overall isotropic temperature factor, until no further corrections other than individual temperature factor adjustments were indicated. At this point the discrepancy factor, R, was 13% for the $hk0$ zone and 14% for the $h0l$ zone. Further work on the structure was suspended until eighteen months later when, with an IBM-709 and the program of Busing & Levy (1959) available to us, the refinement was completed by the full-matrix least-squares method in three dimensions.

In the least-squares computations a weighting scheme similar to Hughes' (1941) was employed. The initial R factor was 25% for three dimensional data, using the coordinates previously obtained by refinement in two projections. Two least-squares refinement cycles on positions and isotropic temperature factors, followed by one cycle on anisotropic temperature factors alone, resulted in a reduction of R to 7·6%. A difference-Fourier synthesis was computed at this stage to locate hydrogen atoms. Of a total of 16 hydrogen atoms in the asymmetric unit, only 10 were actually located unambiguously. These had an average peak height of 0·42 e.Å^{-3} and a range of 0·28 to 0·57 e.Å^{-3}. Since each of these hydrogen atom locations is geometrically reasonable, and since the estimated standard deviation of the electron density at this stage was only 0·13 e.Å^{-3} as calculated from the formula $\sigma(\varrho)=(1/V)[\Sigma(\Delta|F|)^2]^{\frac{1}{2}}$ (Cruickshank, 1949), it is very likely that there actually are hydrogen atoms in these positions. The missing hydrogen atoms were the two on the water molecule, one on the phosphate group and one each on C6, C9 and C15.

Table 1. *Positional parameters of non-hydrogen atoms, in fractional coordinates* $\times 10^4$, *and their estimated standard deviations*

	x/a	y/b	z/c
P1	1866 ± 2	8000 ± 0	10263 ± 5
O2	2503 ± 5	9025 ± 5	11165 ± 15
O3	1255 ± 5	8057 ± 7	7586 ± 14
O4	1073 ± 6	7680 ± 7	12498 ± 16
O5	2645 ± 5	6937 ± 5	10014 ± 14
C6	3361 ± 8	6756 ± 7	12423 ± 21
C7	3997 ± 6	5691 ± 7	11899 ± 19
C8	4388 ± 6	5481 ± 7	9046 ± 18
C9	4591 ± 6	4195 ± 7	9219 ± 20
C10	3640 ± 6	3788 ± 7	10667 ± 19
O11	3325 ± 5	4704 ± 5	12411 ± 14
O12	5246 ± 5	6138 ± 6	8324 ± 14
O13	5488 ± 5	3972 ± 6	11017 ± 15
N14	2735 ± 5	3534 ± 6	8732 ± 16
C15	1924 ± 7	4253 ± 7	7819 ± 22
N16	1339 ± 5	3789 ± 6	5805 ± 17
C17	1755 ± 6	2737 ± 6	5479 ± 18
C18	2643 ± 6	2554 ± 7	7214 ± 19
N19	3249 ± 6	1629 ± 6	7429 ± 18
C20	2970 ± 6	850 ± 7	5629 ± 23
N21	2170 ± 6	939 ± 6	3689 ± 17
C22	1501 ± 7	1837 ± 7	3553 ± 21
N23	722 ± 6	1885 ± 7	1718 ± 19
O24	263 ± 7	228 ± 7	−2322 ± 20

The ten hydrogen atoms whose locations were now established were next introduced into the structure and given the temperature factors of the atoms to which they were covalently bonded. Least-squares refinement was then continued with the following parameters being adjusted in subsequent cycles; non-hydrogen atom positions, non-hydrogen atom temperature factors, non-hydrogen atom positions (final positional adjustment, cycle 6), non-hydrogen atom temperature factors (final temperature factor adjustment, cycle 7) and finally three cycles (8 through 10) of hydrogen atom positions. The mean final adjustment in each type of parameter, in terms of their standard deviations, was 0·12σ for cycle 6, 0·07σ for cycle 7 and 0·19σ for cycle 10. The maximum adjustment in each case was 0·45σ, 0·28σ and 0·74σ.

Table 2. *Thermal parameters of non-hydrogen atoms and their estimated standard deviations*

β as given here is defined by:

$$T=\exp\{-10^{-4}(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)\}$$

	β_{11}	β_{22}	β_{33}	β_{12}	β_{12}	β_{23}
P 1	20 ± 1	33 ± 1	134 ± 11	1 ± 1	−6 ± 3	−14 ± 3
O 2	42 ± 5	35 ± 5	305 ± 40	−2 ± 4	4 ± 10	−40 ± 10
O 3	37 ± 4	69 ± 6	184 ± 34	18 ± 5	−14 ± 9	−2 ± 12
O 4	44 ± 5	89 ± 8	260 ± 41	−30 ± 4	−6 ± 11	−29 ± 12
O 5	42 ± 5	22 ± 4	261 ± 38	6 ± 3	−38 ± 10	−27 ± 9
C 6	46 ± 7	37 ± 7	226 ± 55	−3 ± 5	−44 ± 15	4 ± 13
C 7	23 ± 5	33 ± 6	165 ± 49	2 ± 4	−6 ± 11	−9 ± 12
C 8	19 ± 4	30 ± 6	150 ± 47	0 ± 4	−11 ± 11	−11 ± 11
C 9	18 ± 5	49 ± 7	199 ± 48	1 ± 4	−14 ± 11	−38 ± 13
C10	28 ± 5	28 ± 6	207 ± 50	−2 ± 4	−16 ± 12	−24 ± 12
O11	35 ± 4	34 ± 5	224 ± 37	−11 ± 3	9 ± 9	−14 ± 9
O12	38 ± 5	55 ± 5	184 ± 37	−14 ± 4	−13 ± 10	17 ± 10
O13	21 ± 4	57 ± 6	312 ± 39	10 ± 4	−31 ± 10	2 ± 11
N14	22 ± 4	29 ± 5	201 ± 44	−2 ± 3	2 ± 10	−23 ± 10
C15	28 ± 6	38 ± 7	325 ± 57	−2 ± 5	−28 ± 14	−8 ± 14
N16	24 ± 4	29 ± 5	335 ± 49	7 ± 4	−30 ± 11	−10 ± 12
C17	27 ± 5	31 ± 6	125 ± 43	1 ± 4	−11 ± 11	−10 ± 11
C18	28 ± 5	36 ± 6	158 ± 47	6 ± 4	−1 ± 12	−16 ± 12
N19	28 ± 5	30 ± 5	313 ± 47	4 ± 4	−7 ± 11	10 ± 12
C20	27 ± 5	22 ± 6	415 ± 60	5 ± 5	−5 ± 15	3 ± 14
N21	27 ± 5	36 ± 5	271 ± 48	−2 ± 4	2 ± 12	−14 ± 12
C22	25 ± 5	43 ± 7	209 ± 51	−4 ± 4	−18 ± 13	−9 ± 13
N23	31 ± 5	55 ± 7	269 ± 48	6 ± 4	−23 ± 12	−45 ± 13
O24	80 ± 8	72 ± 7	521 ± 58	−14 ± 6	−94 ± 17	−53 ± 15

Table 3. *Positional parameters of hydrogen atoms, in fractional coordinates* $\times 10^3$, *and their estimated standard deviations*

	x/a	y/b	z/c
H25	379 ± 10	752 ± 10	1276 ± 26
H26	454 ± 9	560 ± 9	1338 ± 27
H27	386 ± 8	572 ± 9	734 ± 24
H28	386 ± 8	291 ± 10	1134 ± 23
H29	559 ± 9	616 ± 10	976 ± 26
H30	610 ± 9	406 ± 10	1006 ± 27
H31	322 ± 9	16 ± 10	599 ± 29
H32	193 ± 9	24 ± 9	267 ± 27
H33	18 ± 10	234 ± 10	233 ± 26
H34	40 ± 9	124 ± 10	28 ± 27
H35	468	390	715

A single scale factor was included throughout the refinement as an adjustable parameter.

A concluding round of structure factor calculations resulted in an R factor of 6·8% for the 1197 reflections with measurable intensities. The refined atomic parameters are given in Tables 1, 2 and 3. Observed and calculated structure factors are listed in Table 4.

A final difference-Fourier synthesis was computed from these structure factors in an attempt to locate the six missing hydrogen atoms. A well defined positive region, of peak height 0·36 e.Å^{-3} was found at the position expected for the hydrogen atom attached to C9. Its parameters are listed opposite H35 in Table 3. No other prominent peaks in stereochemically plausible locations could be found, although several others of about the same height as H35 were present.

Table 4. *Observed and calculated structure factors*

Within each group of constant k and l, the columns contain from left to right: h, $10F_o$, $10F_c$ and the phase angle in millicycles. An asterisk indicates the estimated minimum observable $10F_o$ for reflections which were too weak to be measured

H,0,0

1	97	130	500
2	529	560	500
3	313	297	500
4	20*	25	000
5	125	122	500
6	127	114	000
7	139	118	000
8	110	106	500
9	72	66	999
10	85	78	500
11	193	190	000
12	36*	35	500
13	116	109	500
14	122	116	500
15	145	143	000
16	64	70	000

H,1,0

1	49	91	938
2	432	416	661
3	143	157	275
4	209	206	930
5	289	287	708
6	25*	23	737
7	186	168	855
8	50	55	053
9	90	67	929
10	51	51	469
11	81	87	607
12	182	177	401
13	76	64	500
14	79	76	816
15	125	134	947
16	42	51	112

H,2,0

0	334	336	466
1	517	543	443
2	495	468	971
3	526	492	036
4	283	276	986
5	225	216	823
6	26*	16	998
7	101	91	633
8	85	81	541
9	166	158	886
10	93	95	256
11	273	242	561
12	105	111	255
13	162	162	021
14	53	82	047
15	72	50	506
16	126	110	556

H,3,0

1	491	476	933
2	344	337	950
3	303	298	125
4	248	250	654
5	279	274	424
6	60	61	509
7	281	282	995
8	248	257	487
9	180	179	580
10	37*	19	029
11	215	219	845
12	72	64	739
13	63	62	197
14	85	86	373
15	82	76	625

H,4,0

0	251	263	273
1	478	444	857
2	60	66	991
3	316	304	610
4	162	150	373
5	301	274	995
6	51	41	809
7	92	82	036
8	84	81	379
9	85	82	499
10	250	269	994
11	76	64	959
12	175	143	678
13	145	138	423
14	64	70	354
15	92	72	469

H,5,0

1	36	34	367
2	478	494	508
3	469	455	411
4	78	81	977
5	348	340	000
6	189	201	605
7	166	164	697
8	76	80	457
9	111	111	002
10	55	40	057
11	85	77	474
12	79	75	576
13	52	44	798
14	63	59	026
15	38	48	042

H,6,0

0	144	150	147
1	208	206	649
2	121	108	012
3	343	354	826
4	159	150	113
5	125	118	273
6	316	319	551
7	177	185	974
8	176	173	967
9	38*	42	287
10	195	193	426
11	78	74	276
12	102	103	659
13	98	96	039
14	105	123	908

H,7,0

1	228	226	019
2	208	193	936
3	127	120	558
4	226	226	690
5	96	95	440
6	35*	24	437
7	79	57	358
8	37*	34	318
9	47	42	419
10	87	86	628
11	91	87	094
12	91	92	037
13	64	69	510
14	62	63	616

H,8,0

0	297	291	952
1	98	94	934
2	114	129	670
3	177	175	421
4	92	75	495
5	155	157	981
6	57	63	911
7	38*	16	755
8	194	196	533
9	49	53	770
10	73	74	204
11	94	105	902
12	43	51	005
13	53	43	424

H,9,0

1	252	271	505
2	70	69	876
3	102	107	084
4	111	106	784
5	190	188	274
6	57	32	637
7	38*	18	241
8	108	109	683
9	62	53	623
10	49	43	011
11	46	49	209
12	118	131	481
13	91	99	506

H,10,0

0	302	314	382
1	76	75	530
2	176	171	831
3	216	207	083
4	144	161	704
5	93	98	633
6	50	42	406
7	47	56	034
8	72	53	020
9	49	19	949
10	40	24	635
11	59	57	582
12	64	61	142

H,11,0

1	213	218	108
2	96	82	024
3	172	176	465
4	101	92	556
5	67	66	961
6	36	33	543
7	56	63	055
8	46	46	101
9	83	78	466
10	86	91	469
11	33	32	721

H,12,0

0	47	43	769
1	78	74	737
2	56	44	969
3	86	89	424
4	94	92	534
5	68	76	041
6	65	55	971
7	39	19	426
8	42	28	668
9	53	54	209
10	18	6	192

H,13,0

1	81	72	427
2	108	105	584
3	32	9	679
4	148	145	079
5	32	26	579
6	59	49	528
7	83	93	480
8	37	24	548

H,14,0

0	67	58	734
1	58	51	492
2	43	45	016
3	44	29	811
4	79	63	053
5	74	66	430
6	85	90	572

H,0,1

-15	112	126	000
-14	89	107	500
-13	47*	6	500
-12	79	106	500
-11	206	227	000
-10	85	92	500
-9	65	77	500
-8	295	309	500
-7	163	141	500
-6	79	60	000
-5	30	10	500
-4	353	346	000
-3	82	88	000
-2	36	41	000
-1	305	335	500
0	704	740	000
1	466	497	500
2	158	175	000
3	212	194	500
4	207	235	000
5	219	235	000
6	377	375	500
7	38*	40	000
8	109	115	000
9	45*	9	998
10	48*	17	501
11	294	324	000
12	104	118	000
13	179	193	500
14	86	99	500

H,1,1

-15	116	121	503
-14	86	95	798
-13	146	150	011
-12	62	50	083
-11	112	116	602
-10	111	97	464
-9	130	130	296
-8	96	117	681
-7	92	89	110
-6	168	175	178
-5	45	48	633
-4	491	498	560
-3	757	793	307
-2	734	817	030
-1	576	626	897
0	239	227	446
1	487	503	437
2	150	164	815
3	438	470	197
4	304	288	880
5	111	106	922
6	189	167	184
7	156	148	440
8	90	101	453
9	92	97	019
10	48*	21	423
11	62	77	609
12	161	168	415
13	81	79	821
14	142	137	941
15	78	77	047

H,2,1

-15	66	64	243
-14	55	59	460
-13	112	125	833
-12	49*	42	354
-11	96	106	503
-10	112	108	516
-9	139	135	953
-8	95	102	055
-7	89	78	150
-6	195	181	582
-5	68	67	464
-4	394	401	633
-3	397	397	307
-2	484	497	143
-1	364	366	179
0	381	376	621
1	263	267	448
2	491	523	917
3	292	298	805
4	187	197	234
5	248	241	720
6	114	104	049
7	179	175	917
8	140	143	098
9	98	87	732
10	151	146	343
11	186	191	631
12	118	131	077
13	67	69	041
14	104	117	040
15	39	39	484

H,3,1

-15	102	100	096
-14	71	73	948
-13	104	110	386
-12	91	96	366
-11	50*	36	872
-10	135	147	888
-9	121	115	868
-8	93	90	173
-7	314	322	464
-6	120	115	512
-5	53	62	973
-4	431	426	059
-3	163	183	588
-2	107	133	529
-1	240	270	251
0	251	267	059
1	327	328	197
2	315	306	032
3	199	207	203
4	111	118	492
5	209	221	378
6	132	128	859
7	192	192	056
8	79	66	529
9	262	274	655
10	194	218	357
11	114	106	946
12	118	128	943
13	76	72	943
14	63	72	374
15	97	101	474

H,4,1

-15	44	39	427
-14	46	38	711
-13	60	56	451
-12	48*	20	005
-11	151	159	142
-10	147	147	850
-9	156	165	483
-8	190	185	479
-7	89	70	893
-6	269	272	102
-5	296	288	145
-4	437	451	544
-3	189	206	606
-2	83	82	369
-1	273	286	779
0	169	181	393
1	263	267	932
2	160	164	129
3	181	167	430
4	182	195	240
5	229	246	886
6	201	201	866
7	43*	39	551
8	45*	25	574
9	140	145	882
10	132	140	006
11	124	129	417
12	48*	32	189
13	105	124	554
14	37*	47	726
15	52	66	932

H,5,1

-15	30*	12	870
-14	43	52	613
-13	42*	33	392
-12	109	106	851
-11	62	56	238
-10	162	163	393
-9	113	122	586
-8	79	69	127
-7	144	155	018
-6	374	377	954
-5	185	194	487
-4	36*	21	228
-3	174	175	754
-2	268	252	243
-1	72	74	097
0	267	264	070
1	305	293	319
2	227	222	465
3	247	231	785
4	211	210	026
5	184	186	137
6	152	143	573
7	210	216	490
8	157	172	129
9	335	377	938
10	65	65	015
11	192	190	470
12	106	103	495
13	64	61	481
14	34*	22	586
15	86	92	007

H,6,1

-14	59	65	842
-13	85	85	147
-12	45*	52	609
-11	68	60	195
-10	108	116	469
-9	108	117	193
-8	141	142	005
-7	110	123	609
-6	147	152	477
-5	122	140	417
-4	122	114	948
-3	178	186	927
-2	246	236	159
-1	217	213	558
0	95	89	823
1	209	201	601
2	210	204	275
3	242	240	907
4	121	120	838
5	42*	25	785
6	242	252	478
7	194	203	065
8	48*	24	098
9	50*	52	088
10	187	194	631
11	127	113	795
12	70	65	026
13	50	61	030
14	36*	43	889

H,7,1

-14	31*	10	082
-13	50	51	406
-12	113	121	551
-11	74	69	912
-10	135	138	052
-9	135	143	953
-8	116	126	486
-7	202	219	521
-6	66	82	293
-5	193	193	033
-4	215	216	081
-3	93	81	007
-2	201	204	270
-1	156	162	642
0	159	158	818
1	200	185	796
2	111	110	068
3	112	98	254
4	84	68	275
5	64	67	268
6	233	246	005
7	58	32	536
8	118	115	489
9	49*	25	253
10	140	112	439
11	45*	25	020
12	143	130	962
13	57	67	014
14	73	80	520

H,8,1

-13	30*	44	608
-12	37*	20	318
-11	51	29	790
-10	70	61	368
-9	74	76	783
-8	50*	10	375
-7	159	164	359
-6	49*	44	152
-5	119	135	998
-4	99	100	930
-3	202	206	476
-2	116	114	671
-1	168	162	217
0	219	217	982
1	44	49	056
2	132	139	481
3	117	119	614
4	47*	54	635
5	87	91	948
6	55	59	620
7	127	116	907
8	212	225	488
9	68	77	816
10	112	108	963
11	126	129	040
12	88	81	264
13	63	88	624

H,9,1

-13	20*	40	884
-12	32*	34	110
-11	39*	29	924
-10	100	114	475
-9	63	63	508
-8	127	137	053
-7	130	134	927
-6	103	110	338
-5	99	95	712
-4	117	115	361
-3	194	206	556
-2	132	131	865
-1	373	407	994
0	143	130	244
1	117	127	420
2	48*	18	956
3	132	129	102
4	143	154	869
5	72	65	566
6	125	131	599
7	72	65	123
8	93	82	249
9	45*	51	189
10	42*	40	053
11	76	78	723
12	135	130	455

H,10,1

-12	22*	45	391
-11	52	57	506
-10	49	51	076
-9	42*	49	931
-8	45*	44	025
-7	48*	56	881
-6	49*	45	440
-5	136	143	526
-4	62	40	518
-3	246	251	120
-2	83	68	032
-1	124	132	428
0	151	164	582
1	135	139	843
2	49*	52	030
3	50*	29	320
4	50*	54	582
5	49*	58	208
6	106	124	734
7	86	96	860
8	63	70	148
9	39*	28	049
10	46	55	598
11	163	146	471

H,11,1

-11	21*	19	249
-10	30*	33	925
-9	36*	3	955
-8	55	23	285
-7	74	73	606
-6	49	32	653
-5	72	63	015
-4	127	131	995
-3	74	67	807
-2	72	66	389
-1	59	54	265
0	156	168	474
1	205	213	953
2	154	150	904
3	85	99	426
4	71	76	434
5	106	121	106
6	42*	43	313
7	95	101	822
8	40*	40	856
9	35*	54	519
10	53	45	502

H,12,1

-9	53	46	402
-8	107	81	416
-7	45	45	772
-6	101	105	092
-5	117	112	103
-4	45*	47	840
-3	153	154	603
-2	132	131	501
-1	47*	35	026
0	128	123	000
1	47*	50	463
2	47*	36	289
3	45*	27	853
4	71	54	621
5	93	87	069
6	40*	29	934
7	81	87	425
8	130	130	603
9	31*	31	225

H,13,1

-7	67	69	973
-6	33*	11	828
-5	61	64	348
-4	62	44	490
-3	77	91	869
-2	41*	34	899
-1	67	60	146
0	42*	12	007
1	42*	28	580
2	87	90	513
3	40*	28	906
4	96	87	946
5	35*	3	988
6	32*	27	220
7	94	76	520

H,14,1

-5	109	119	510
-4	123	127	006
-3	91	67	065
-2	33*	31	924
-1	33*	33	690
0	97	30	082
1	105	95	407
2	39*	16	753
3	55	51	857
4	33*	12	417
5	70	65	553

H,0,2

-15	88	93	500
-14	71	60	500
-13	97	92	000
-12	55	101	500
-11	196	198	000
-10	83	87	500
-9	178	178	500
-8	127	109	500
-7	44*	15	500
-6	108	101	000
-5	136	123	000
-4	402	369	000
-3	288	308	500
-2	185	131	500
-1	14*	24	999
0	296	279	500
1	201	234	500
2	266	221	500
3	320	292	500
4	30*	3	500
5	380	390	000
6	166	144	500
7	144	138	000
8	151	156	500
9	167	158	500
10	108	83	000
11	191	185	000
12	48*	1	500
13	122	118	500
14	68	81	000

H,1,2

-15	70	64	499
-14	43*	45	478
-13	95	110	050
-12	53*	54	942
-11	71	90	616
-10	178	165	447
-9	114	112	607
-8	66	44	149
-7	173	158	972
-6	211	205	074
-5	75	72	011
-4	339	302	601
-3	325	317	507
-2	232	237	862
-1	212	201	143
0	111	96	785
1	225	220	434
2	370	368	024
3	376	368	010
4	179	167	697
5	446	414	343
6	129	124	480
7	198	199	498
8	93	103	308
9	219	239	109
10	123	117	984
11	89	89	802
12	110	110	515
13	43*	29	518
14	131	109	063

H,2,2

-15	39*	40	727
-14	67	61	986
-13	79	71	910
-12	121	119	436
-11	58	62	326
-10	115	103	605
-9	173	178	882
-8	239	235	946
-7	206	206	173
-6	421	396	544
-5	420	408	368
-4	137	128	159
-3	187	180	183
-2	290	301	690
-1	84	68	360
0	272	267	599
1	224	218	119
2	233	216	984
3	165	146	764
4	300	272	400
5	400	381	550
6	132	136	385
7	202	185	884
8	139	149	051
9	75	76	987
10	174	159	256
11	133	130	517
12	47*	37	849
13	74	73	784
14	102	101	006

H,3,2

-15	72	75	179
-14	40*	57	748
-13	47*	40	657
-12	68	78	476
-11	54*	23	852
-10	157	151	016
-9	52*	61	886
-8	202	201	407
-7	242	224	679
-6	265	249	662
-5	204	196	454
-4	387	364	142
-3	178	172	020
-2	352	341	816
-1	270	273	213
0	28*	35	172
1	179	165	504
2	125	114	870
3	206	201	323
4	141	130	384
5	53	41	284
6	237	234	955
7	96	95	021
8	58	55	891
9	174	169	628
10	147	152	313
11	92	84	078
12	155	152	166
13	41*	32	507
14	33*	32	708

H,4,2

-14	45*	31	475
-13	45*	25	529
-12	113	110	893
-11	143	120	046
-10	55*	11	813
-9	160	151	328
-8	275	270	484
-7	48*	20	566
-6	262	251	990
-5	173	154	795
-4	229	204	547
-3	245	226	249
-2	230	219	524
-1	66	52	283
0	200	197	209
1	110	100	314
2	230	216	487
3	109	101	460
4	159	151	899
5	234	229	021
6	328	320	021
7	309	306	487
8	375	376	498
9	50*	19	878
10	154	152	956
11	48*	28	747
12	76	78	054
13	95	94	566
14	30*	37	433

H,5,2

-14	79	75	501
-13	100	76	172
-12	113	105	680
-11	52*	6	388
-10	104	92	497
-9	101	88	563
-8	53*	38	314
-7	278	283	923
-6	139	139	988
-5	252	226	473
-4	97	83	697
-3	41*	46	423
-2	200	183	351
-1	81	87	565
0	38*	9	642
1	50	27	290
2	97	74	151
3	139	121	862
4	275	266	985
5	61	49	086
6	119	113	345
7	119	115	590
8	186	181	040
9	103	93	254
10	67	67	199
11	79	86	726
12	43*	25	225
13	36*	8	399
14	67	53	750

H,6,2

-13	120	111	212
-12	73	89	455
-11	63	95	600
-10	116	114	660
-9	176	183	071
-8	163	155	032
-7	110	121	563
-6	51	71	200
-5	319	316	517
-4	70	70	987
-3	122	100	031
-2	89	70	070
-1	147	148	407
0	98	90	074
1	141	138	583
2	62	67	281
3	265	246	151
4	223	223	554
5	194	185	504
6	247	251	335
7	217	211	025
8	281	280	810
9	91	85	004
10	153	146	501
11	97	88	600
12	40*	38	088
13	32*	43	047

H,7,2

-13	40*	19	613
-12	82	87	575
-11	47	45	180
-10	51*	16	918
-9	146	123	921
-8	79	89	366
-7	110	116	448
-6	115	115	305
-5	73	59	993
-4	115	106	764
-3	92	79	717
-2	48*	43	931
-1	229	225	607
0	101	97	059
1	198	179	036
2	96	87	128
3	100	98	512
4	138	114	450
5	102	100	571
6	260	246	033
7	196	186	071
8	50*	36	187
9	126	111	589
10	46*	63	480
11	105	95	951
12	36*	20	228
13	26*	31	057

H,8,2

-12	36*	38	922
-11	111	113	053
-10	63	46	339
-9	154	144	583
-8	53*	37	433
-7	118	103	344
-6	164	155	899
-5	93	106	945
-4	238	234	098
-3	296	303	402
-2	139	119	705
-1	51	42	764
0	67	62	026
1	169	175	317
2	135	127	465
3	48	49	749
4	130	122	156
5	132	131	817
6	98	109	551
7	58	62	967
8	101	111	433
9	60	54	190
10	60	56	140
11	108	99	991
12	29*	9	473

H,9,2

-11	37*	25	032
-10	89	80	514
-9	86	89	568
-8	51*	22	381
-7	78	81	924
-6	64	59	127
-5	117	111	626
-4	135	128	530
-3	54*	68	207
-2	200	192	046
-1	118	80	117
0	137	129	623
1	70	52	617
2	115	102	602
3	179	183	005
4	168	173	078
5	85	82	496
6	140	129	568
7	48*	15	116
8	62	63	369
9	97	95	877
10	129	133	109
11	71	64	820

H,10,2

-10	36*	27	978
-9	84	96	851
-8	49	51	166
-7	49*	32	233
-6	51*	36	340
-5	100	91	449
-4	67	74	384
-3	55*	60	872
-2	145	145	980
-1	145	137	318
0	182	169	516
1	74	75	999
2	145	143	019
3	121	126	733
4	49*	45	314
5	99	93	283
6	72	69	467
7	45*	30	272
8	79	71	033
9	52	53	829
10	95	76	456

H,11,2

-10	75*	60	994
-9	84	56	036
-8	40	37	404
-7	111	102	567
-6	47*	35	537
-5	49*	32	058
-4	98	89	014
-3	52*	23	119
-2	79	75	541
-1	53*	13	584
0	66	60	454
1	135	136	022
2	66	56	458
3	51	28	235
4	68	65	630
5	86	88	811
6	67	57	067
7	80	69	980
8	35*	11	426
9	97	96	636

H,12,2

-8	67	73	406
-7	108	82	690
-6	69	80	996
-5	43*	29	007
-4	70	72	386
-3	110	115	628
-2	48*	42	254
-1	111	109	079
0	124	120	954
1	58	29	569
2	134	134	388
3	55	61	363
4	41*	22	957
5	113	119	980
6	36*	8	930
7	49	52	394
8	77	80	645

H,13,2

-6	68	55	114
-5	95	94	601
-4	55	59	482
-3	39*	40	302
-2	40*	14	783
-1	41*	11	789
0	41*	24	925
1	38*	14	851
2	64	60	517
3	35*	25	076
4	33*	48	916
5	54	40	911

H,14,2

-2	58	50	981
-1	33*	40	569
0	72	57	378
1	26*	15	269
2	25*	34	813

H,0,3

-14	44*	51	500
-13	45	34	500
-12	51*	3	500
-11	55	70	000
-10	56*	8	501
-9	56*	14	000
-8	53*	1	500
-7	50*	80	500
-6	279	306	000
-5	127	104	000
-4	36*	44	500
-3	321	290	500
-2	55	62	500
-1	162	134	000
0	51*	40	500
1	48	73	000
2	388	406	500
3	105	113	000
4	46	2	999
5	159	169	000
6	166	176	500
7	52*	45	000
8	73	95	500
9	56*	33	500
10	55*	32	000
11	52*	5	993
12	47*	21	500
13	92	102	500

H,1,3

-14	61	41	436
-13	77	66	997
-12	98	105	925
-11	68	63	174
-10	173	186	380
-9	149	161	680
-8	67	44	118
-7	56	71	887
-6	70	61	200
-5	97	87	678
-4	119	121	585
-3	97	109	863
-2	195	189	017
-1	62	63	128
0	262	267	703
1	278	276	506
2	30*	30	438
3	233	226	952
4	75	83	140
5	137	134	293
6	154	161	603
7	177	187	499
8	55*	56	709
9	187	201	971
10	125	132	998
11	63	71	309
12	83	85	558
13	50*	36	356

H,2,3

-14	85	86	988
-13	56	67	893
-12	50*	44	585
-11	54*	40	558
-10	72	53	534
-9	99	96	052
-8	60	57	044
-7	183	180	264
-6	198	190	685
-5	64	62	346
-4	66	71	141
-3	99	99	059
-2	286	285	800

Table 4 (*cont.*)

H,2,3			
-1	91	81	345
0	187	191	402
1	156	143	000
2	164	171	655
3	68	70	861
4	141	139	312
5	141	146	513
6	150	155	357
7	56	36	128
8	102	105	131
9	56*	39	801
10	116	121	621
11	105	95	433
12	46	45	184
13	49*	33	942
H,3,3			
-14	55*	8	804
-13	53	56	498
-12	86	79	491
-11	53*	61	746
-10	79	67	967
-9	86	77	918
-8	131	131	420
-7	91	79	496
-6	49	53	334
-5	182	234	919
-4	100	99	989
-3	105	102	427
-2	268	279	623
-1	132	141	333
0	102	103	089
1	180	187	983
2	147	139	750
3	231	222	510
4	220	221	509
5	274	293	755
6	242	246	962
7	158	158	159
8	102	128	277
9	97	113	498
10	55*	25	975
11	51*	27	839
12	71	77	061
13	64*	32	550
H,4,3			
-13	79	84	408
-12	46*	18	929
-11	93	108	934
-10	91	82	141
-9	75	66	114
-8	224	231	621
-7	85	78	637
-6	145	140	199
-5	49	62	934
-4	63	65	752
-3	43*	62	412
-2	41*	4	735
-1	166	160	173
0	99	111	845
1	106	94	107
2	116	107	438
3	171	175	484
4	135	131	852
5	206	227	184
6	71	72	894
7	214	237	549
8	102	104	439
9	98	98	342
10	53	42	955
11	49*	43	985
12	42*	17	507
H,5,3			
-13	109	98	082
-12	43	60	100
-11	49*	27	548
-10	101	99	583
-9	56*	38	292
-8	94	102	926
-7	222	220	881
-6	223	213	394
-5	128	141	408
-4	66	82	558
-3	91	87	304
-2	94	107	862
-1	113	109	882
0	182	177	529
1	136	139	682
2	47*	4	337
3	88	98	266
4	254	253	961
5	125	130	833
6	241	239	370
7	179	173	534
8	160	169	912
9	55*	23	183
10	51*	55	003
11	72	69	546
12	74	55	352
H,6,3			
-13	93	83	977
-12	37	33	423
-11	91	93	552
-10	51*	69	530
-9	63	68	064
-8	56	62	991
-7	111	110	717
-6	85	100	038
-5	129	136	471
-4	136	147	030
-3	73	74	739
-2	50*	3	242
-1	121	134	398
0	102	105	586
1	50*	33	246
2	147	147	028
3	244	252	175
4	131	143	496
5	109	109	568
6	160	158	631
7	67	63	048
8	142	137	009
9	55	48	299
10	55	44	424
11	42*	37	315
12	49	37	778
H,7,3			
-12	82	87	624
-11	53	60	151
-10	48*	52	159
-9	52	73	998
-8	58	34	195
-7	112	123	442
-6	132	142	510
-5	199	192	844
-4	152	155	878
-3	146	146	338
-2	97	79	536
-1	132	135	563
0	53	42	015
1	107	116	113
2	76	77	887
3	141	146	564
4	175	183	423
5	90	79	095
6	126	127	955
7	95	97	760
8	53*	18	300
9	82	85	471
10	44*	45	468
11	86	99	966
H,8,3			
-11	85	91	029
-10	43*	28	963
-9	71	74	480
-8	52*	13	773
-7	54*	39	035
-6	79	83	972
-5	84	80	484
-4	79	85	869
-3	143	156	476
-2	95	89	152
-1	93	98	970
0	99	103	052
1	127	125	395
2	235	251	619
3	144	142	736
4	164	189	216
5	113	109	061
6	120	117	093
7	52*	11	182
8	96	93	398
9	45*	35	424
10	38*	40	708
11	64*	9	011
H,9,3			
-10	64	70	497
-9	42*	49	557
-8	47*	20	481
-7	51	45	914
-6	75	80	960
-5	85	90	291
-4	81	72	456
-3	56	57	768
-2	108	122	868
-1	124	132	244
0	90	90	655
1	132	127	424
2	83	73	514
3	146	147	893
4	55*	29	364
5	53*	12	363
6	62	58	368
7	90	86	541
8	44*	20	460
9	154	153	897
10	86	87	131
H,10,3			
-9	55	56	107
-8	40*	43	055
-7	45*	24	886
-6	72	84	429
-5	70	77	445
-4	52*	55	009
-3	69	76	911
-2	54*	63	869
-1	101	109	601
0	73	81	500
1	85	83	137
2	151	165	048
3	52	61	959
4	80	86	368
5	97	109	573
6	46*	16	181
7	42*	31	402
8	74	68	147
9	62*	30	944
H,11,3			
-8	64	74	278
-7	95	76	607
-6	45	48	778
-5	67	56	170
-4	47*	39	193
-3	48*	33	425
-2	114	109	526
-1	49*	46	357
0	82	81	958
1	97	93	965
2	71	66	434
3	47*	38	437
4	102	119	540
5	72	68	716
6	85	84	031
7	76*	51	844
H,12,3			
-5	34*	28	851
-4	38*	19	318
-3	77	58	700
-2	72	65	276
-1	43*	52	137
0	55	43	964
1	42	29	883
2	51*	37	460
3	58*	33	420
4	85*	22	583
5	87	79	085
H,13,3			
-2	68*	79	977
-1	79	62	676
0	72*	43	492
H,0,4			
-12	105	112	000
-11	62	55	000
-10	57	68	000
-9	60*	38	500
-8	60*	1	500
-7	59*	3	504
-6	220*	236	000
-5	147	166	500
-4	168	171	500
-3	70	73	500
-2	38*	40	000
-1	247	234	000
0	187	156	000
1	26*	16	998
2	279	307	500
3	186	178	000
4	51	58	500
5	172	211	000
6	52*	40	000
7	95	93	500
8	133	128	500
9	58	40	000
10	108	112	000
11	56*	9	500
H,1,4			
-12	43*	46	095
-11	52*	37	123
-10	62	71	541
-9	59*	31	641
-8	86	104	141
-7	59*	65	828
-6	56*	3	376
-5	79	69	566
-4	131	129	471
-3	64	72	010
-2	190	174	015
-1	154	150	919
0	50	50	568
1	213	209	359
2	166	166	481
3	101	98	744
4	46*	35	508
5	128	102	891
6	64	49	087
7	95	92	833
8	60	52	841
9	63*	29	009
10	58*	31	781
11	96	91	490
H,2,4			
-12	42*	15	348
-11	82	76	406
-10	56*	30	640
-9	59*	20	963
-8	66	64	128
-7	100	113	315
-6	143	142	669
-5	62	56	788
-4	182	183	017
-3	134	139	046
-2	63	65	794
-1	194	197	585
0	178	183	409
1	48	50	944
2	118	115	926
3	257	270	044
4	165	161	370
5	157	160	596
6	67	40	913
7	79	83	040
8	70	59	092
9	56	64	404
10	147	130	602
11	95	75	362
H,3,4			
-12	82	87	441
-11	48*	40	750
-10	55*	42	993
-9	58*	54	070
-8	60*	16	605
-7	60*	30	551
-6	59*	32	229
-5	189	176	023
-4	53*	31	323
-3	49*	39	505
-2	171	172	484
-1	85	99	420
0	130	132	782
1	173	175	972
2	60	39	541
3	102	110	726
4	95	82	359
5	83	80	704
6	68	72	223
7	60	59	922
8	52*	60	493
9	110	113	527
10	78	68	933
11	76	68	112
H,4,4			
-12	33*	20	162
-11	83	80	914
-10	52*	48	354
-9	118	102	510
-8	59	46	606
-7	143	149	934
-6	50*	44	137
-5	58*	42	054
-4	56*	61	486
-3	108	196	533
-2	102	112	106
-1	197	185	052
0	208	225	837
1	85	87	967
2	94	91	341
3	177	179	473
4	134	151	737
5	89	95	156
6	54*	31	189
7	110	104	435
8	66	71	863
9	58*	23	791
10	81	84	013
11	51*	43	947
H,5,4			
-11	44	39	873
-10	62	69	744
-9	71	69	374
-8	116	102	323
-7	60	51	044
-6	60*	39	557
-5	60*	23	618
-4	105	115	503
-3	77	72	950
-2	135	133	936
-1	132	135	144
0	93	97	540
1	116	123	622
2	77	74	507
3	93	99	099
4	150	149	001
5	90	91	597
6	142	153	484
7	55	55	610
8	49*	47	030
9	55*	42	946
10	46	33	801
H,6,4			
-11	46	45	395
-10	54*	33	097
-9	140	144	808
-8	63	62	069
-7	79	83	351
-6	83	89	467
-5	76	73	628
-4	164	162	988
-3	107	109	872
-2	59	62	165
-1	109	111	364
0	201	201	533
1	53*	38	177
2	109	122	932
3	108	91	996
4	98	98	694
5	60	37	170
6	67	73	738
7	59	52	047
8	125	108	045
9	139	135	488
10	49*	47	336
H,7,4			
-10	98	96	134
-9	56*	37	735
-8	137	103	715
-7	55*	31	451
-6	58*	36	191
-5	59*	61	916
-4	60*	55	894
-3	85	106	303
-2	121	125	570
-1	60*	40	531
0	115	118	973
1	152	160	233
2	54*	15	892
3	74	75	740
4	127	130	554
5	52*	52	278
6	49*	13	211
7	78	81	797
8	67	40	341
9	53	52	412
H,8,4			
-9	45*	46	456
-8	55*	21	504
-7	50*	21	186
-6	109	96	915
-5	56*	42	228
-4	58*	63	418
-3	127	134	539
-2	59	39	659
-1	89	114	876
0	122	124	997
1	53*	24	777
2	165	164	492
3	61	60	100
4	71	62	958
5	86	80	886
6	84	73	158
7	104	95	496
8	36	47	389
H,9,4			
-8	42*	35	877
-7	90	89	040
-6	58*	44	847
-5	93	91	420
-4	53*	32	442
-3	55*	40	068
-2	66	89	850
-1	93	109	169
0	67	71	694
1	136	147	508
2	60	54	521
3	72	85	144
4	63	67	235
5	87	78	791
6	83	54	468
H,10,4			
-6	56	53	577
-5	68	71	746
-4	62	51	901
-3	88	90	097
-2	61*	8	064
-1	74	90	546
0	62	40	372
1	91	95	641
2	46	50	931
3	67	50	215
4	47*	37	385
5	68	65	568
H,11,4			
-4	56*	39	923
-3	64	30	617
-2	84	78	548
-1	86	79	326
0	49*	47	123
1	79	73	844
2	53*	53	897
3	58	64	459

The most notable feature of the final difference-Fourier map is a positive peak of height 0·75 e.Å^{-3} located exactly halfway between pairs of phosphorus atoms related by the c translation. This is most probably a result of diffraction effects caused by truncation of the data beyond $l=4$.

Results and discussion

The crystal structure of adenosine-5′-phosphate, viewed in orthographic projection along the c-axis, is illustrated in Fig. 1. The ten hydrogen atoms H25–H34 located in the first difference-Fourier synthesis are shown with their final coordinates as computed by the least-squares refinement. Hydrogen atom H35 is shown according to its location in the final difference map. Hydrogen atoms not actually found are omitted. Presumed hydrogen bonds are indicated by dashed lines.

In Fig. 2 a single molecule is shown in projection along the glycosidic bond C10–N14. The unit cell b-axis is horizontal to the left in this drawing. Hydrogen bonded atoms of neighboring molecules are indicated by light circles. These atoms are also labelled with atom identification numbers in Fig. 1. Bond lengths and angles for covalent bonds not involving hydrogen atoms are given in Tables 5 and 6. Hydrogen-bond lengths and angles are listed in Tables

Table 5. *Bond lengths of covalent bonds not involving hydrogen atoms, and their estimated standard deviations*

Bond	Length
P1–O2	1·514 ± 0·008 Å
P1–O3	1·495 ± 0·008
P1–O4	1·566 ± 0·008
P1–O5	1·610 ± 0·007
O5–C6	1·475 ± 0·012
C6–C7	1·525 ± 0·013
C7–C8	1·520 ± 0·012
C8–C9	1·544 ± 0·012
C8–O12	1·400 ± 0·011
C9–C10	1·509 ± 0·013
C9–O13	1·438 ± 0·011
C10–O11	1·445 ± 0·011
C7–O11	1·476 ± 0·011
C10–N14	1·492 ± 0·012
N14–C15	1·398 ± 0·012
N14–C18	1·377 ± 0·012
C15–N16	1·328 ± 0·012
N16–C17	1·364 ± 0·011
C17–C18	1·403 ± 0·012
C18–N19	1·341 ± 0·012
N19–C20	1·312 ± 0·012
C20–N21	1·368 ± 0·012
N21–C22	1·362 ± 0·012
C22–C17	1·448 ± 0·013
C22–N23	1·312 ± 0·013

Table 6. *Bond angles for covalent bonds not involving hydrogen atoms, and their estimated standard deviations*

Angle	Degrees
O2–P1–O3	118·2 ± 0·4°
O2–P1–O4	110·2 ± 0·4
O2–P1–O5	108·7 ± 0·4
O3–P1–O4	106·9 ± 0·4
O3–P1–O5	105·7 ± 0·4
O4–P1–O5	106·5 ± 0·4
P1–O5–C6	114·7 ± 0·5
O5–C6–C7	107·6 ± 0·7
C6–C7–C8	119·1 ± 0·7
C6–C7–O11	107·9 ± 0·7
C8–C7–O11	104·0 ± 0·6
C7–C8–C9	99·9 ± 0·7
C7–C8–O12	115·5 ± 0·7
C9–C8–O12	115·4 ± 0·7
C8–C9–C10	101·7 ± 0·6
C8–C9–O13	110·0 ± 0·6
C10–C9–O13	107·0 ± 0·6
C9–C10–O11	106·7 ± 0·6
C9–C10–N14	112·6 ± 0·7
O11–C10–N14	107·2 ± 0·7
C7–O11–C10	108·3 ± 0·7
C10–N14–C15	128·6 ± 0·7
C10–N14–C18	123·8 ± 0·7
C15–N14–C18	107·1 ± 0·7
N14–C15–C16	111·8 ± 0·8
C15–N16–C17	104·7 ± 0·7
N16–C17–C18	112·3 ± 0·7
N16–C17–C22	131·9 ± 0·8
C18–C17–C22	115·6 ± 0·7
N14–C18–C17	104·1 ± 0·7
N14–C18–N19	127·4 ± 0·8
C17–C18–N19	128·5 ± 0·8
C18–N19–C20	112·3 ± 0·8
N19–C20–N21	125·7 ± 0·8
C20–N21–C22	122·8 ± 0·8
C17–C22–N21	114·7 ± 0·8
C17–C22–N23	123·7 ± 0·8
N21–C22–N23	121·5 ± 0·8

7 and 8. Table 9 contains the bond lengths of covalent bonds involving hydrogen atoms.

The adenine ring

As in adenine hydrochloride (Cochran, 1951), the adenine ring is protonated at N21. Bond lengths and angles also agree in general with those found in adenine hydrochloride. Possibly significant bond-length discrepancies, however, are in N14–C15, where we find 1·398 ± 0·012 Å in comparison with Cochran's 1·33 ± 0·01 Å, and in C22–C17 where our length is 1·448 ± 0·013 Å whereas Cochran finds 1·40 ± 0·01 Å. Other differences are 0·03 Å or less. The greatest discrepancy in bond angles is at N14–C15–N16 for which we obtain 111·8 ± 0·8° as against 115° found in adenine hydrochloride. Possibly even these differences would be still smaller had not Cochran assumed exact planarity of the adenine ring in computing atomic y-coordinates.

Interestingly, we find small but probably significant deviations from exact planarity in the adenine ring of this structure. The nine ring atoms have an r.m.s. deviation of 0·026 Å from the least-squares plane through them; in particular C17 is 0·043 Å below this plane and N21 is 0·051 Å above it. It is evident from Fig. 2 that this distortion must be due to the 25°

Table 7. *Hydrogen-bond lengths and their estimated standard deviations*

Bond	Length
O3–O4′	2·525 ± 0·011 Å
O12–N19′	2·828 ± 0·012
H29–N19′	2·1 ± 0·1
O13–O2′	2·819 ± 0·011
H30–O2′	1·9 ± 0·1
N21–O2″	2·619 ± 0·011
H32–O2″	1·8 ± 0·1
N23–O3′	2·901 ± 0·012
H33–O3′	2·0 ± 0·1
N23–O24	2·824 ± 0·014
H34–O24	1·7 ± 0·1
O24–O3″	2·863 ± 0·013

Table 8. *Hydrogen-bond angles and their estimated standard deviations*

Angle	Degrees
P1–O2–O13′	111·0 ± 0·4°
P1–O2–N21″	137·1 ± 0·4
O13′–O2–N21″	111·4 ± 0·3
P1–O3–O4′	151·1 ± 0·5
P1–O3–N23′	107·4 ± 0·4
P1–O3–O24′	104·0 ± 0·4
O4′–O3–N23′	89·2 ± 0·3
O4′–O3–O24′	98·7 ± 0·3
N23′–O3–O24′	92·2 ± 0·3
P1–O4–O3‴	127·4 ± 0·4
C8–O12–N19′	116·4 ± 0·5
H29–O12–N19′	15·0 ± 5·0
C9–O13–O2′	118·7 ± 0·5
H30–O13–O2′	9·0 ± 5·0
C18–N19–O12′	126·7 ± 0·6
C20–N19–O12′	120·3 ± 0·6
C20–N21–O2″	97·4 ± 0·6
C22–N21–O2″	139·8 ± 0·6
H32–N21–O2″	27·0 ± 5·0
C22–N23–O3′	125·2 ± 0·6
O24–N23–O3′	104·9 ± 0·4
C22–N23–O24	125·2 ± 0·7
H33–N23–O3′	15·0 ± 5·0
H34–N23–O24	10·0 ± 5·0
N23–O24–O3″	123·6 ± 0·4

Table 9. *Bond lengths of covalent bonds involving hydrogen atoms*

Standard deviations are approximately 0·1 Å

Bond	Length
C6–H25	1·1 Å
C7–H26	1·0
C8–H27	1·1
C9–H35	1·1
C10–H28	1·1
O12–H29	0·8
O13–H30	0·9
C20–H31	0·9
N21–H32	1·0
N23–H33	0·9
N23–H34	1·1

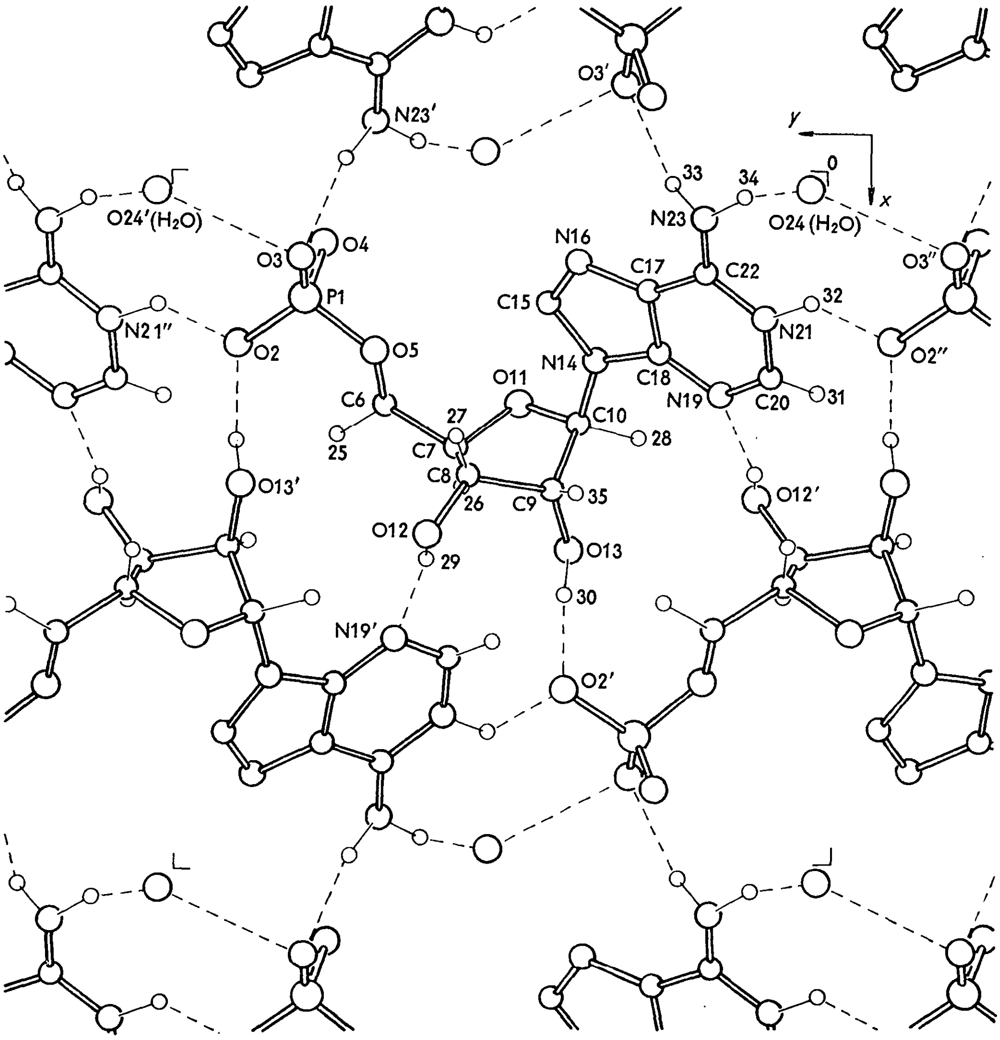


Fig. 1. Orthographic projection along the c-axis of the crystal structure of adenosine-5′-phosphate.

rotation from the adenine plane experienced by the plane of the hydrogen-bond pair formed by N23.

Even more drastic is the deviation of the glycosidic bond C10–N14 from the plane of the adenine ring, with C10 lying 0·211 Å above and forming an angle of 8° with the least-squares plane. This is clearly seen in Fig. 2. The effect is much greater than in calcium thymidylate (Trueblood, Horn & Luzzati, 1961) where the displacement of the C1′ atom of the ribose ring (corresponding to our C10) from the plane of the thymine ring was 0·050 Å. It is not clear whether forces arising out of the requirement for efficient hydrogen bonding and molecular packing would be sufficient to account for this deformation, but that is the most likely explanation. The glycosidic bond has a length of 1·492 ± 0·012 Å, in reasonable agreement with 1·466 ± 0·018 Å for the corresponding bond in calcium thymidylate and with the standard C–N single bond length of 1·47 Å.

The ribose ring

The ribose ring is best described as puckered in such a way that atom C8 (C3′ in the usual terminology) lies 0·66 Å above the approximate plane formed by the remaining four ring atoms. These four atoms have an r.m.s. deviation of 0·022 Å from the least-squares plane through them. When planes are fitted to any of the other four groups of four ring atoms the corresponding r.m.s. deviation is always 0·1–0·2 Å.

The same conformation is also observed for 2′-deoxyribose in calcium thymidylate as well as for the unphosphorylated ribose of cytidine (Furberg, 1950). However in cytidylic acid-*b*, where the 3′ oxygen rather than the 5′ oxygen carries the phosphate group, Alver & Furberg (1959) report the C2′ atom to be 0·5 Å out of the plane. Spencer (1959*a*) has reviewed the question of puckering in the furanose ring.

Bond lengths and angles in the ribose ring have their expected values; there is also excellent agreement with the lengths and angles reported for the deoxyribose of calcium thymidylate, showing that the replacement of an OH group by a hydrogen atom at position 2′ has essentially no effect upon the structure. In fact, the r.m.s. deviation among corresponding interatomic distances in the two sugars is 0·019 Å, or about the same as the estimated standard deviations of these quantities reported by Trueblood, Horn & Luzatti, with the greatest difference amounting to about 2σ. A similar assertion may be made about the bond angles.

The phosphate group

Bond lengths and angles of the phosphate group fall within the expected range. Since we have not unequivocally located the required hydrogen atom, some uncertainty remains concerning which oxygen atom belongs to the unionized OH function. In fact, a small peak of height 0·27 e.Å^{-3} is actually observed in the final difference-Fourier map at a distance of 0·9 Å from atom O4, but it is 24° away from the line joining the presumably hydrogen bonded atoms O4 and O3‴ (in the molecule below) and therefore we did not feel confident in identifying it as a hydrogen atom covalently bonded to O4. Nevertheless, as the following discussion will bring out, it is likely that the oxygen atom of the unionized OH function is indeed O4.

There have been to date only three precise determinations of structures containing the organic phosphate group: dibenzyl phosphoric acid by Dunitz & Rollett (1956), calcium thymidylate by Trueblood, Horn & Luzzati (1961) and 2-amino-ethanol phosphate by Kraut (1961). Comparison of bond lengths and angles in these structures leads to these conclusions: (1) the P–O*R* distance (where *R* is an organic residue or a hydrogen atom) is 1·565 Å, with an r.m.s. deviation among six reported values of 0·018 Å, whereas the P–O distance when no other atom is covalently bonded to the oxygen is 1·490 Å, with an r.m.s. deviation of 0·016 Å among six reported values; (2) the shorter the P–O bonds the stronger their mutual repulsion, so that an O–P–O angle of about 117° is to be expected between the two shortest bonds; (3) the oxygen atom of a P–O*R* group does not act as a hydrogen bond acceptor whereas the P–O oxygen atom may accept one, two or even three hydrogen bonds. The provisional nature of these conclusions should be emphasized, particularly (2) and (3), for they are based on a minimum of experimental data and must await the availability of much more information about organic phosphate structures for their verification. However, the assumption that O4 belongs to the unionized OH group is then in complete accord with each of these hypotheses and further strengthens them.

The question of hydrogen bonding at the phosphate group will be further discussed below.

Conformation of the molecule

In describing the molecular conformation of nucleotides it is useful to have a standard way of referring to the relative orientation of hererocyclic base and ribose moiety about the glycosidic bond. For this reason Donohue & Trueblood (1960) have introduced the torsion angle $\varphi_{\rm CN}$, defined as the angle formed by the trace of the plane of the base with the projection of the C1′–O bond when the projection is taken along the glycosidic bond itself. This projection is shown for adenosine-5′-phosphate in Fig. 2. It may be compared with Fig. 2 of Donohue & Trueblood (1960) where similar projections are shown for thymidylic acid residues in two proposed *DNA* structures and for the thymidylate ion. It has been pointed out by Spencer (1959*b*), by Donohue & Trueblood (1960) and by Trueblood, Horn & Luzzati (1961) that thus far only pyrimidine nucleosides and nucleotides have been studied, and that these all fall in the *anti* range of conformation—that is, with $\varphi_{\rm CN}$ in the neighborhood of $-30°$ (as contrasted with $\varphi_{\rm CN} = ca.$ 150° for the *syn* conformation). As seen in Fig. 2, $\varphi_{\rm CN}$ in adenosine-5′-phosphate is about $-18°$; it is thus in the *anti* conformation and suggests that purine nucleotides may also prefer this conformation.

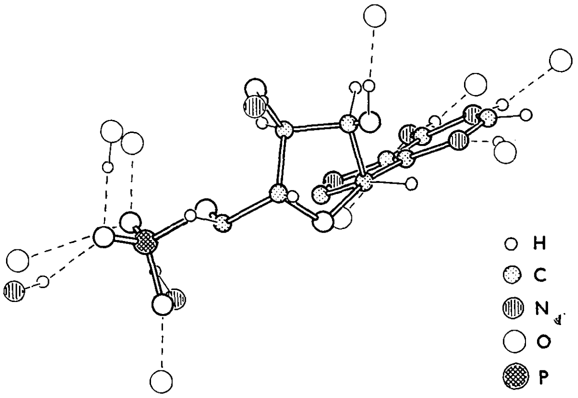


Fig. 2. Orthographic projection along the glycosidic bond C10–N14 of a single molecule of adenosine-5′-phosphate.

The plane of the ribose ring is at a dihedral angle of 76° to the adenine plane, in agreement with the corresponding angle of 76° in cytidine and 75° in calcium thymidylate. It differs appreciably, however, from the dihedral angle in cytidylic acid-*b*, which we have computed to be 62° from the positional parameters given by Alver & Furberg (1959). This disagreement is not surprising in view of the differing ribose conformations in the two compounds.

Rich *et al.* (1961) have recently proposed a structure for fibers of polyadenylic acid and have published details of the bond lengths and angles assumed in their model. We have compared these with the parameters of adenosine-5′-phosphate and observe generally good agreement. The largest and possibly significant discrepancies are found for the bond lengths C8–O12 (+0·07 Å), N14–C15 (−0·07 Å) and C22–C17 (−0·06 Å), and for the angles C6–C7–C8 (−7°), C9–C10–N14 (−7°) and P1–O5–C6 (+6°).

Hydrogen bonds

Presumed hydrogen bonds are shown by dashed lines in Figs. 1 and 2. Where the location of the hydrogen atom within a hydrogen bond is not clear in the difference-Fourier maps, the dashed line is simply drawn between two hydrogen bonded atoms and the hydrogen atom is omitted. In Fig. 1 the hydrogen bonds are not shown between atoms O4 and O3‴ in the molecule below, or between O3 and O4′ in the molecule above, since they lie almost perpendicular to the plane of projection. They are shown, however, in Fig. 2.

The hydrogen-bond distribution about the phosphate group presents some interesting features which were touched upon in the above discussion. Atom O2 is the acceptor of two hydrogen bonds which are in an approximately planar trigonal arrangement with its covalent bond to atom P1. This implies a large degree of double-bond character in the P1–O2 bond, in agreement with the observation that its length of 1·514 Å is much less than the expected P–O single-bond length of 1·73 Å (Pauling, 1960). A similar planar trigonal arrangement about the oxygen atom was found for the two short P–O bonds in 2-aminoethanol phosphate (Kraut, 1961). About atom O3, on the other hand, which acts as hydrogen bond acceptor toward atoms O4′, O24′ and N23′, we find a roughly tetrahedral arrangement of bonds; yet the bond length P1–O3 is only 1·495 Å. A similar arrangement is seen about O_{III} in calcium thymidylate. If it is assumed that hydrogen bonds tend to lie in the direction of orbitals on the acceptor atom containing unshared electron pairs, one must conclude that there is something rather unusual about the hybridization of orbitals around an oxygen atom in a P–O bond.

It is noteworthy that several hydrogen bonds of the structure are appreciably non-linear. In particular H32 is 27° away from the line joining N21 with O2″. In spite of this the short H32–O2″ contact of 1·8 Å as well as the geometrical reasonableness of the entire arrangement leaves little doubt as to the reality of this non-linear hydrogen bond. Hamilton (1961) has recently emphasized that the assumption of strict linearity in hydrogen bonds may not always be justified.

Hydrogen bonding at the water molecule represents something of a problem since we have failed to locate either of its hydrogen atoms. Evidently one of them must be pointing in the direction of atom O3″, but there is no other atom sufficiently close to the water oxygen, O24, to provide a second obvious hydrogen bond acceptor. The closest non-bonded atom is N16 in the adjacent unit cell, at a distance of 3·11 Å.

Thermal parameters

The magnitudes and direction cosines of the principal axes of the ellipsoids of thermal vibration are given in Table 10 for those atoms which are significantly anisotropic. The quantities C_{ia}, C_{ib} and C_{ic} are cosines of the angles between the ith principal axis and the a, b and c axes of the unit cell. The B_i are given in Å^2. The criterion for significant anisotropy was arbitrarily chosen to be that at least one of the β_{ij} for the atom

Table 10. *Magnitudes and direction cosines of the principal axes of thermal vibration ellipsoids*

	Axis i	B_i	C_{ia}	C_{ib}	C_{ic}
P1	1	2·03	−0·247	−0·867	0·442
	2	1·39	0·832	−0·418	−0·398
	3	1·04	0·496	0·270	0·804
O2	1	3·47	0·030	−0·521	0·851
	2	2·76	0·999	−0·020	−0·089
	3	1·39	0·042	0·854	0·517
O3	1	4·49	−0·486	−0·870	0·109
	2	2·15	0·650	−0·428	−0·654
	3	1·45	0·584	−0·247	0·748
O4	1	6·04	0·473	−0·872	0·107
	2	2·75	0·513	0·156	−0·865
	3	1·53	0·716	0·464	0·490
O5	1	3·91	−0·721	−0·251	0·676
	2	1·66	−0·692	0·311	−0·622
	3	0·95	0·037	0·917	0·396
C6	1	3·86	−0·814	0·125	0·601
	2	2·07	0·059	0·989	−0·136
	3	1·32	0·578	0·075	0·788
C9	1	3·32	−0·146	−0·826	0·551
	2	1·56	0·567	−0·521	−0·661
	3	0·91	0·811	0·216	0·510
O11	1	2·87	0·721	−0·583	0·345
	2	2·06	0·444	−0·028	−0·914
	3	1·36	0·533	0·812	0·216
O12	1	3·84	−0·584	0·770	0·281
	2	1·89	0·713	0·638	−0·321
	3	1·58	0·388	−0·012	0·905
O13	1	3·55	−0·439	−0·652	0·635
	2	3·16	0·108	−0·721	−0·689
	3	0·89	0·892	−0·234	0·350
N14	1	2·30	0·033	−0·603	0·795
	2	1·44	0·948	−0·233	−0·256
	3	1·20	0·316	0·763	0·550
C15	1	3·53	−0·447	−0·090	0·908
	2	2·18	−0·264	0·964	−0·024
	3	1·44	0·855	0·251	0·418
N16	1	3·66	−0·425	−0·186	0·903
	2	1·73	−0·391	−0·845	−0·348
	3	1·04	0·817	−0·501	0·252
C20	1	3·99	−0·135	0·012	0·996
	2	1·91	−0·892	−0·438	−0·078
	3	1·08	0·432	−0·899	0·051
N23	1	4·12	−0·334	−0·716	0·627
	2	2·07	0·703	−0·622	−0·373
	3	1·51	0·628	0·316	0·684
O24	1	7·78	−0·724	−0·077	0·715
	2	4·88	−0·417	0·840	−0·329
	3	1·75	0·549	0·537	0·617

in question should differ by more than 2σ (see Table 2) from the value it would have if the atom were actually vibrating isotropically with B equal to the mean principal axis B_i. For those atoms which do not meet this requirement only the mean of the principal axis B_i is given in Table 11.

Table 11. *Mean thermal parameters for atoms which are not significantly anisotropic*

Atom	B	Atom	B
C7	1·6 Å^2	C18	1·8 Å^2
C8	1·4	N19	2·2
C10	1·8	N21	2·1
C17	1·6	C22	2·0

It should be emphasized that the physical significance of anisotropic thermal parameters obtained by least-squares refinement must be interpreted with care. For example if the β_{ij} of atom C10 are reduced to principal axes one obtains B_i values of 2·57, 1·82 and 1·03 Å^2. Seemingly C10 is undergoing highly anisotropic thermal vibration. But compare the observed β_{ij} with those equivalent to strictly isotropic vibration with a B value of 1·8 Å^2: $\beta_{ij}=28$, 32, 189, 0, 3 and 0 (in the same order as in Table 2). The largest difference is in β_{23}, and this amounts to only twice the estimated standard deviation of the parameter. Other differences are still less significant. It must be concluded therefore that in spite of the apparently appreciable spread in the B_i of atom C10 obtained from least-squares refinement, the thermal motion of this atom cannot be said to show significant anisotropy.

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References

Alver, A. & Furberg, S. (1959). *Acta Chem. Scand.* **13**, 910.

Brown, D. M., Fasman, G. D., Magrath, D. I., Todd, A. R., Cochran, W. & Woolfson, M. M. (1953). *Nature, Lond.* **172**, 1184.

Busing, W. R. & Levy, H. A. (1959). *A Crystallographic Least Squares Refinement Program for the IBM*-704. U.S. Atomic Energy Commission Publication ORNL 59-4-37.

Cochran, W. (1951). *Acta Cryst.* **4**, 81.

Crick, F. H. C. & Watson, J. D. (1954). *Proc. Roy. Soc.* A, **223**, 80.

Cruickshank, D. W. J. (1949). *Acta Cryst.* **2**, 65.

Donohue, J. & Trueblood, K. N. (1960). *J. Mol. Biol.* **2**, 363.

Dunitz, J. D. & Rollett, J. S. (1956). *Acta Cryst.* **9**, 327.

Furberg, S. (1950). *Acta Cryst.* **3**, 325.

Hamilton, W. C. (1961). *Acta Cryst.* **14**, 95.

Hughes, E. W. (1941). *J. Amer. Chem. Soc.* **63**, 1737.

Kraut, J. & Jensen, L. H. (1960). *Nature, Lond.* **186**, 798.

Kraut, J. (1961). *Acta Cryst.* **14**, 1146.

Langridge, R., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F. & Hamilton, L. D. (1960*a*). *J. Mol. Biol.* **2**, 19.

Langridge, R., Marvin, D. A., Seeds, W. E., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F. & Hamilton, L. D. (1960*b*). *J. Mol. Biol.* **2**, 38.

McWeeny, R. (1951). *Acta Cryst.* **4**, 513.

Pauling, L. (1960). *The Nature of the Chemical Bond*, 3rd edition. Ithaca: Cornell University Press.

Rich, A., Davies, D. R., Crick, F. H. C. & Watson, J. D. (1961). *J. Mol. Biol.* **3**, 71.

Spencer, M. (1959*a*). *Acta Cryst.* **12**, 59.

Spencer, M. (1959*b*). *Acta Cryst.* **12**, 66.

Trueblood, K. N., Horn, P. & Luzzati, V. (1961). *Acta Cryst.* **14**, 965.

Watson, J. D. & Crick, F. H. C. (1953). *Nature, Lond.* **171**, 737.