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## An X-ray Study of the Hydrogen Bonding in the Crystalline L-Arginine Phosphate Monohydrate Complex

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The crystal structure of the L-arginine phosphate monohydrate complex has been determined from 1453 three-dimensional X-ray diffractometer data and refined by least-squares methods to a residual of  $R = 3.9\%$ . The X-ray results suggest that the L-arginine molecule is deprotonated at the carboxyl group and protonated at the guanidyl and amino groups and that the phosphate ion carries one negative charge. The L-arginine molecule is in a less extended conformation than found previously and both the guanidinium group and the carboxyl- $C^\alpha$  system are essentially planar. The crystal structure is interlaced by an intricate hydrogen-bonding scheme with apparently one bifurcated hydrogen bond involving atom  $N^6$ . The symmetrical guanidinium-phosphate ion geometry proposed for the interaction of basic proteins with DNA is not found in the L-arginine-phosphate monohydrate structure. It may therefore be inferred that the symmetrical complex is not necessarily preferred over a more complicated hydrogen bonding arrangement. The same structure has been published recently by Aoki, Nagamo & Iitaka (*Acta Cryst.* (1971). **B27**, 11).

### Introduction

The interaction of basic polypeptides, such as histones and protamines, with nucleic acids is partially governed by ionic bonds between phosphate residues and the basic amino acid side chains of lysine and arginine. Studies with polyamino acid-DNA complexes revealed that the arginine guanidinium-phosphate ion bond is stronger than the charged lysine-phosphate ion bond (Leng & Felsenfeld, 1966; Suwalsky, 1968; Miller & Inbar, 1969). Similar evidence comes from binding studies of nucleotides with basic polyamino acids (Wagner & Arav, 1968; Wagner, 1971). A symmetrical guanidinium-phosphate ion bond enhanced by two hydrogen bonds and a coupled charge resonance has been proposed to explain these facts (Suwalsky, 1968; Lewin, 1969). In order to test this supposition, direct X-ray studies on a crystalline L-arginine phosphate complex, Fig. 1, have been undertaken.

After we had completed this structure it came to our attention that the same study had been undertaken by Aoki, Nagamo & Iitaka (1971). The structural investigations of the Japanese colleagues were based on film data, taken with  $Cu K\alpha$  radiation and measured with a microdensitometer. The final discrepancy index  $R$  which they obtained is 0.095 for 1229 data, the estimated standard deviations of the interatomic distances average  $0.017 \text{ \AA}$  and two out of the nineteen protons were not located. As the data utilized in our work were collected by diffractometer measurement, it was possible to refine the structure somewhat more completely; the estimated standard deviation in bond lengths has been reduced to  $0.004 \text{ \AA}$ .

### Materials and methods

The chloride counterions of anion exchange resin *AG 1-X2* (Bio. Rad. Labs.) were replaced by phosphate ions, the  $pH$  was adjusted to 6 and a column was prepared. L-Arginine.HCl (Calbiochem) dissolved in water was added and the arginine-containing fractions were collected, lyophilized and recrystallized from water. Chemical analysis of the crystal obtained in this way indicated a ratio of one mole of phosphate ion per mole of arginine.

Adjusting the  $pH$  of the anion exchange resin to 8.5 and applying the same procedure as outlined above yielded a compound with a phosphate:arginine ratio of 1:2 which, however, did not crystallize but behaved like a very viscous gel.

A thick, prismatic L-arginine phosphate crystal measuring  $0.2 \times 0.3 \times 0.2 \text{ mm}$  was mounted on a glass fibre along the longest dimension and used for all subsequent X-ray crystallographic investigations.

The monoclinic space group  $P2_1$  and cell parameters were derived by photographic methods and refined diffractometrically to:

$$\begin{aligned} a &= 10.898 \pm 0.003 \text{ \AA} \\ b &= 7.910 \pm 0.002 \\ c &= 7.339 \pm 0.002 \\ \beta &= 97.97 \pm 0.03^\circ; \end{aligned}$$

Systematic extinctions for  $0k0$ ,  $k = \text{odd}$ .

Volume of the unit cell =  $627 \text{ \AA}^3$ .

Molecular weight ( $C_6H_{15}O_2N_4 \cdot H_2PO_4^- \cdot H_2O$ ) = 291.

The density of the crystals, which was determined using the flotation technique ( $D_{\text{obs}} = 1.53 \text{ g.cm}^{-3}$ ), is

in agreement with the density calculated from the crystallographic asymmetric unit of the L-arginine phosphate monohydrate complex assuming two formula units per unit cell ( $D_{\text{calc}} = 1.542 \text{ g.cm}^{-3}$ ).

Using the  $\omega$ ,  $2\theta$  scan technique the intensities of 1453 reflexions were measured by means of an automatic four-circle diffractometer equipped with a Mo X-ray tube (Zr-filtered Mo  $K\alpha$  radiation,  $\lambda = 0.70926 \text{ \AA}$ ). The data were converted to structure factor amplitudes by application of the usual geometrical factors but were not corrected for absorption. A sharpened Patterson function Fourier synthesis revealed the location of the phosphate anion and the remaining atoms were located by standard heavy atom techniques. The structure amplitudes were assigned weights  $1/\sigma_{F_{\text{obs}}}$  according to counting statistics (Stout & Jensen, 1968) and data with  $F_{\text{obs}} < 3\sigma_{F_{\text{obs}}}$  were treated as being unobserved. The atomic positional and thermal parameters were refined by least-squares full-matrix techniques minimizing the quantity  $\sum(|F_{\text{obs}}| - |F_{\text{calc}}|)^2/\sigma_{F_{\text{obs}}}^2$  (Bu-

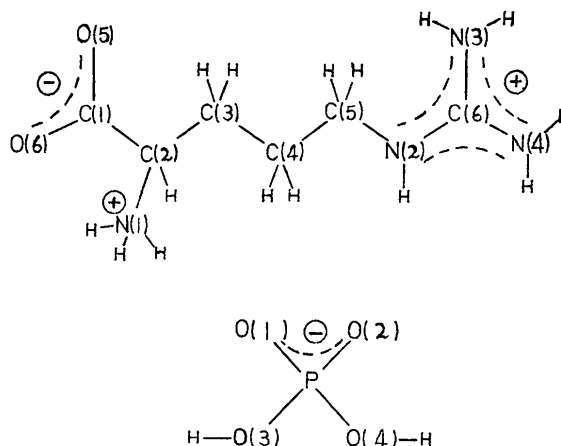


Fig. 1. Chemical formula of the L-arginine phosphate complex and numbering scheme used in the text. The IUPAC nomenclature is C $^{\alpha}$  for C(2), C $^{\beta}$  for C(3), C $^{\gamma}$  for C(4), C $^{\delta}$  for C(5), N $^{\epsilon}$  for N(2), C $^{\eta}$  for C(6), N $^{\eta 1}$  for N(3) and N $^{\eta 2}$  for N(4).

Table 1. Atomic coordinates and thermal parameters

Atomic coordinates are in fractions of the unit-cell axes and thermal parameters are in the form  $T = \exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$ , multiplied by  $10^4$ . The figures in parentheses are the standard deviations of the last digits, estimated from the least-squares variance-covariance matrix.

	x	y	z	$\beta_{11}$	$\beta_{22}$	$\beta_{33}$	$\beta_{12}$	$\beta_{13}$	$\beta_{23}$
P	-118 (0)	7500 (0)	1759 (0)	51 (0)	50 (1)	79 (1)	0 (0)	14 (0)	5 (0)
O(1)	149 (2)	8400 (3)	59 (3)	101 (2)	102 (4)	115 (3)	9 (2)	55 (2)	26 (3)
O(2)	122 (2)	8509 (3)	3491 (2)	69 (2)	74 (3)	102 (3)	-1 (2)	12 (2)	-8 (3)
O(3)	-1531 (2)	6970 (3)	1337 (3)	59 (2)	107 (4)	147 (4)	-13 (2)	-3 (2)	34 (3)
C(4)	638 (2)	5825 (3)	2079 (3)	76 (2)	74 (3)	158 (4)	18 (2)	2 (2)	0 (3)
O(5)	3612 (2)	8354 (3)	9198 (3)	90 (2)	98 (4)	181 (5)	-10 (2)	-40 (3)	8 (4)
O(6)	2367 (2)	9483 (3)	6860 (3)	69 (2)	73 (3)	116 (4)	0 (2)	11 (2)	2 (3)
O(W)	4747 (2)	5850 (4)	1535 (3)	105 (2)	121 (4)	122 (4)	5 (3)	11 (2)	19 (4)
N(1)	769 (2)	6830 (3)	6843 (3)	50 (2)	73 (3)	103 (4)	0 (2)	17 (2)	5 (3)
N(2)	3690 (2)	2242 (4)	4923 (3)	70 (2)	87 (4)	98 (4)	6 (2)	25 (2)	8 (3)
N(3)	2300 (3)	647 (5)	3040 (4)	85 (3)	129 (5)	181 (6)	-30 (3)	28 (3)	1 (5)
N(4)	3175 (2)	2938 (4)	1859 (3)	74 (2)	148 (6)	105 (4)	-40 (3)	-10 (2)	20 (4)
C(1)	2751 (3)	8284 (4)	7908 (4)	56 (2)	72 (4)	98 (5)	3 (2)	18 (2)	-17 (4)
C(2)	2116 (2)	6567 (4)	7515 (4)	52 (2)	66 (4)	91 (5)	6 (2)	17 (2)	2 (3)
C(3)	2701 (3)	5552 (4)	6085 (4)	59 (2)	79 (4)	85 (4)	5 (2)	13 (2)	-13 (4)
C(4)	3952 (2)	4801 (4)	6858 (4)	51 (2)	103 (5)	95 (5)	4 (3)	14 (2)	-12 (4)
C(5)	4486 (2)	3693 (4)	5470 (4)	45 (2)	109 (5)	105 (5)	2 (3)	23 (2)	-14 (4)
C(6)	3064 (2)	1957 (4)	3288 (4)	43 (2)	96 (5)	117 (5)	0 (2)	26 (2)	0 (4)
H(1)	2135 (40)	5974 (67)	8681 (55)	42	79	94	0	8	0
H(2)	336 (36)	7533 (75)	7784 (55)	45	83	99	0	9	0
H(3)	282 (40)	5901 (67)	6637 (55)	45	83	99	0	9	0
H(4)	641 (36)	7579 (74)	5874 (54)	45	83	99	0	9	0
H(5)	2769 (39)	6244 (69)	4915 (54)	46	87	103	0	9	0
H(6)	2117 (40)	4672 (68)	5588 (58)	46	87	103	0	9	0
H(7)	4675 (41)	5699 (71)	7289 (58)	52	96	114	0	10	0
H(8)	3901 (39)	4109 (69)	8020 (59)	52	96	114	0	10	0
H(9)	4577 (41)	4333 (69)	4444 (65)	49	92	109	0	10	0
H(10)	5337 (40)	3336 (70)	5971 (62)	49	92	109	0	10	0
H(11)	3687 (37)	1484 (68)	5650 (56)	51	95	113	0	10	0
H(12)	2277 (48)	169 (79)	3712 (68)	76	143	169	0	15	0
H(13)	1886 (47)	560 (83)	2031 (72)	76	143	169	0	15	0
H(14)	3655 (42)	3618 (73)	2085 (61)	63	117	139	0	13	0
H(15)	2639 (41)	2681 (72)	971 (61)	63	117	139	0	13	0
H(16)	5214 (46)	5253 (78)	1181 (67)	70	130	154	0	14	0
H(17)	4374 (47)	6551 (75)	810 (64)	70	130	154	0	14	0
H(18)	-1795 (44)	6251 (68)	2143 (61)	58	108	128	0	12	0
H(19)	382 (43)	4994 (78)	1045 (64)	64	120	143	0	13	0

Table 2. Observed and calculated structure factors multiplied by 10

Table with 48 columns (H, K, L, Fobs, Fcalc) and 100 rows of numerical data representing observed and calculated structure factors.

Table 2 (cont.)

H	K	L	FOBS	FCAL	H	K	L	FOBS	FCAL	H	K	L	FOBS	FCAL	H	K	L	FOBS	FCAL	H	K	L	FOBS	FCAL	H	K	L	FOBS	FCAL	H	K	L	FOBS	FCAL
7	2	1	157	151	8	0	-1	65	61	9	0	-1	167	169	10	2	-2	177	176	11	1	-1	68	69	12	1	0	16	16	18	18			
7	2	1	176	175	8	1	-2	105	106	9	0	-1	21	20	10	-2	-5	78	78	11	1	-2	65	63	12	1	-1	60	60	19	19			
7	2	1	168	169	8	1	-3	87	86	9	0	-1	4	4	10	0	-1	84	84	11	1	-2	64	61	12	1	-1	62	62	20	20			
7	2	1	172	171	8	1	-4	120	118	9	0	-1	11	11	10	0	-1	72	72	11	1	-2	60	60	12	1	-1	59	59	21	21			
7	2	1	173	176	8	1	-5	151	154	9	0	-1	18	18	10	0	-1	14	12	11	1	-1	66	66	12	1	-1	56	56	22	22			
7	2	1	175	177	8	1	-6	182	181	9	0	-1	25	27	10	0	-1	21	21	11	1	-1	64	64	12	1	-1	54	54	23	23			
7	2	1	177	179	8	1	-7	213	211	9	0	-1	32	31	10	0	-1	28	28	11	1	-1	62	62	12	1	-1	52	52	24	24			
7	2	1	179	181	8	1	-8	244	242	9	0	-1	39	38	10	0	-1	35	35	11	1	-1	60	60	12	1	-1	50	50	25	25			
7	2	1	181	183	8	1	-9	275	273	9	0	-1	46	45	10	0	-1	42	42	11	1	-1	58	58	12	1	-1	48	48	26	26			
7	2	1	183	185	8	1	-10	306	304	9	0	-1	53	52	10	0	-1	49	49	11	1	-1	56	56	12	1	-1	46	46	27	27			
7	2	1	185	187	8	1	-11	337	335	9	0	-1	60	59	10	0	-1	56	56	11	1	-1	54	54	12	1	-1	44	44	28	28			
7	2	1	187	189	8	1	-12	368	366	9	0	-1	67	66	10	0	-1	63	63	11	1	-1	52	52	12	1	-1	42	42	29	29			
7	2	1	189	191	8	1	-13	399	397	9	0	-1	74	73	10	0	-1	70	70	11	1	-1	50	50	12	1	-1	40	40	30	30			
7	2	1	191	193	8	1	-14	430	428	9	0	-1	81	80	10	0	-1	77	77	11	1	-1	48	48	12	1	-1	38	38	31	31			
7	2	1	193	195	8	1	-15	461	459	9	0	-1	88	87	10	0	-1	84	84	11	1	-1	46	46	12	1	-1	36	36	32	32			
7	2	1	195	197	8	1	-16	492	490	9	0	-1	95	94	10	0	-1	91	91	11	1	-1	44	44	12	1	-1	34	34	33	33			
7	2	1	197	199	8	1	-17	523	521	9	0	-1	102	101	10	0	-1	98	98	11	1	-1	42	42	12	1	-1	32	32	34	34			
7	2	1	199	201	8	1	-18	554	552	9	0	-1	109	108	10	0	-1	105	105	11	1	-1	40	40	12	1	-1	30	30	35	35			
7	2	1	201	203	8	1	-19	585	583	9	0	-1	116	115	10	0	-1	112	112	11	1	-1	38	38	12	1	-1	28	28	36	36			
7	2	1	203	205	8	1	-20	616	614	9	0	-1	123	122	10	0	-1	119	119	11	1	-1	36	36	12	1	-1	26	26	37	37			
7	2	1	205	207	8	1	-21	647	645	9	0	-1	130	129	10	0	-1	126	126	11	1	-1	34	34	12	1	-1	24	24	38	38			
7	2	1	207	209	8	1	-22	678	676	9	0	-1	137	136	10	0	-1	133	133	11	1	-1	32	32	12	1	-1	22	22	39	39			
7	2	1	209	211	8	1	-23	709	707	9	0	-1	144	143	10	0	-1	140	140	11	1	-1	30	30	12	1	-1	20	20	40	40			
7	2	1	211	213	8	1	-24	740	738	9	0	-1	151	150	10	0	-1	147	147	11	1	-1	28	28	12	1	-1	18	18	41	41			
7	2	1	213	215	8	1	-25	771	769	9	0	-1	158	157	10	0	-1	154	154	11	1	-1	26	26	12	1	-1	16	16	42	42			
7	2	1	215	217	8	1	-26	802	800	9	0	-1	165	164	10	0	-1	161	161	11	1	-1	24	24	12	1	-1	14	14	43	43			
7	2	1	217	219	8	1	-27	833	831	9	0	-1	172	171	10	0	-1	168	168	11	1	-1	22	22	12	1	-1	12	12	44	44			
7	2	1	219	221	8	1	-28	864	862	9	0	-1	179	178	10	0	-1	175	175	11	1	-1	20	20	12	1	-1	10	10	45	45			
7	2	1	221	223	8	1	-29	895	893	9	0	-1	186	185	10	0	-1	182	182	11	1	-1	18	18	12	1	-1	8	8	46	46			
7	2	1	223	225	8	1	-30	926	924	9	0	-1	193	192	10	0	-1	189	189	11	1	-1	16	16	12	1	-1	6	6	47	47			
7	2	1	225	227	8	1	-31	957	955	9	0	-1	200	199	10	0	-1	196	196	11	1	-1	14	14	12	1	-1	4	4	48	48			
7	2	1	227	229	8	1	-32	988	986	9	0	-1	207	206	10	0	-1	203	203	11	1	-1	12	12	12	1	-1	2	2	49	49			
7	2	1	229	231	8	1	-33	1019	1017	9	0	-1	214	213	10	0	-1	210	210	11	1	-1	10	10	12	1	-1	0	0	50	50			
7	2	1	231	233	8	1	-34	1050	1048	9	0	-1	221	220	10	0	-1	217	217	11	1	-1	8	8	12	1	-1	0	0	51	51			
7	2	1	233	235	8	1	-35	1081	1079	9	0	-1	228	227	10	0	-1	224	224	11	1	-1	6	6	12	1	-1	0	0	52	52			
7	2	1	235	237	8	1	-36	1112	1110	9	0	-1	235	234	10	0	-1	231	231	11	1	-1	4	4	12	1	-1	0	0	53	53			
7	2	1	237	239	8	1	-37	1143	1141	9	0	-1	242	241	10	0	-1	238	238	11	1	-1	2	2	12	1	-1	0	0	54	54			
7	2	1	239	241	8	1	-38	1174	1172	9	0	-1	249	248	10	0	-1	245	245	11	1	-1	0	0	12	1	-1	0	0	55	55			
7	2	1	241	243	8	1	-39	1205	1203	9	0	-1	256	255	10	0	-1	252	252	11	1	-1	0	0	12	1	-1	0	0	56	56			
7	2	1	243	245	8	1	-40	1236	1234	9	0	-1	263	262	10	0	-1	259	259	11	1	-1	0	0	12	1	-1	0	0	57	57			
7	2	1	245	247	8	1	-41	1267	1265	9	0	-1	270	269	10	0	-1	266	266	11	1	-1	0	0	12	1	-1	0	0	58	58			
7	2	1	247	249	8	1	-42	1298	1296	9	0	-1	277	276	10	0	-1	273	273	11	1	-1	0	0	12	1	-1	0	0	59	59			
7	2	1	249	251	8	1	-43	1329	1327	9	0	-1	284	283	10	0	-1	280	280	11	1	-1	0	0	12	1	-1	0	0	60	60			
7	2	1	251	253	8	1	-44	1360	1358	9	0	-1	291	290	10	0	-1	287	287	11	1	-1	0	0	12	1	-1	0	0	61	61			
7	2	1	253	255	8	1	-45	1391	1389	9	0	-1	298	297	10	0	-1	294	294	11	1	-1	0	0	12	1	-1	0	0	62	62			
7	2	1	255	257	8	1	-46	1422	1420	9	0	-1	305	304	10	0	-1	301	301	11	1	-1	0	0	12	1	-1	0	0	63	63			
7	2	1	257	259	8	1	-47	1453	1451	9	0	-1	312	311	10	0	-1	308	308	11	1	-1	0	0	12	1	-1	0	0	64	64			
7	2	1	259	261	8	1	-48	1484	1482	9	0	-1	319	318	10	0	-1	315	315	11	1	-1	0	0	12	1	-1	0	0	65	65			
7	2	1	261	263	8	1	-49	1515	1513	9	0	-1	326	325	10	0	-1	322	322	11	1	-1	0	0	12	1	-1	0	0	66	66			
7	2	1	263	265	8	1	-50	1546	1544	9	0	-1	333	332	10	0	-1	329	329	11	1	-1	0	0	12	1	-1	0	0	67	67			
7	2	1	265	267	8	1	-51	1577	1575	9	0	-1	340	339	10	0	-1	336	336	11	1	-1	0	0	12	1	-1	0	0	68	68			
7	2	1	267	269	8	1	-52	1608	1606	9	0	-1	347	346	10	0	-1	343	343	11	1	-1	0	0	12	1	-1	0	0	69	69			
7	2	1	269	271	8	1	-53	1639	1637	9	0	-1	354	353	10	0	-1	350	350	11	1	-1	0	0	12	1	-1	0	0	70	70			
7	2	1	271	273	8	1	-54	1670	1668	9	0	-1	361	360	10	0	-1	357	357	11	1	-1	0	0	12	1	-1	0	0	71	71			
7	2	1	273	275	8	1	-55	1701	1699	9	0	-1	368																					

C(5)–N(2) has been found to be *trans* in all L-arginine structures investigated crystallographically but *gauche* in the phosphate complex and in some high molecular proteins (see also Aoki *et al.*, 1971). It was also inferred from theoretical considerations (Ponnuswamy, Lakshminarayanan & Sasisekharan, 1971) that the C(3)–C(4)–C(5)–N(2) dihedral angle can assume not only the *trans* conformation but ethane-like staggered *gauche* conformations as well, corresponding to a less extended hydrocarbon chain than in the *trans* form.

(b) *Geometry of the phosphate group*

The bond distances within the phosphate group indicate the charge distribution and the location of the hydrogen atoms attached to two of the oxygen atoms,

O(3) and O(4). The P–O bonds involving the oxygen atoms carrying the negative charge, O(1) and O(2), are shorter ( $\Delta/\sigma=30$ ) than the P–O bonds of the two P–O–H groups and the angle between them, O(1)–P–O(2),  $115.0^\circ$ , is greater than the other O–P–O angles (Fig. 2). One may assume, therefore, that the phosphate ion has the structure illustrated in Fig. 2 (Corbridge, 1966).

(c) *Hydrogen bonding*

The hydrogen bonds deduced for this structure on the basis of located hydrogen atoms and short intermolecular distances are indicated by dashed lines in Figs. 3 and 4, and their geometrical data are listed in Table 3. In Fig. 6 some of the atoms of the complex are

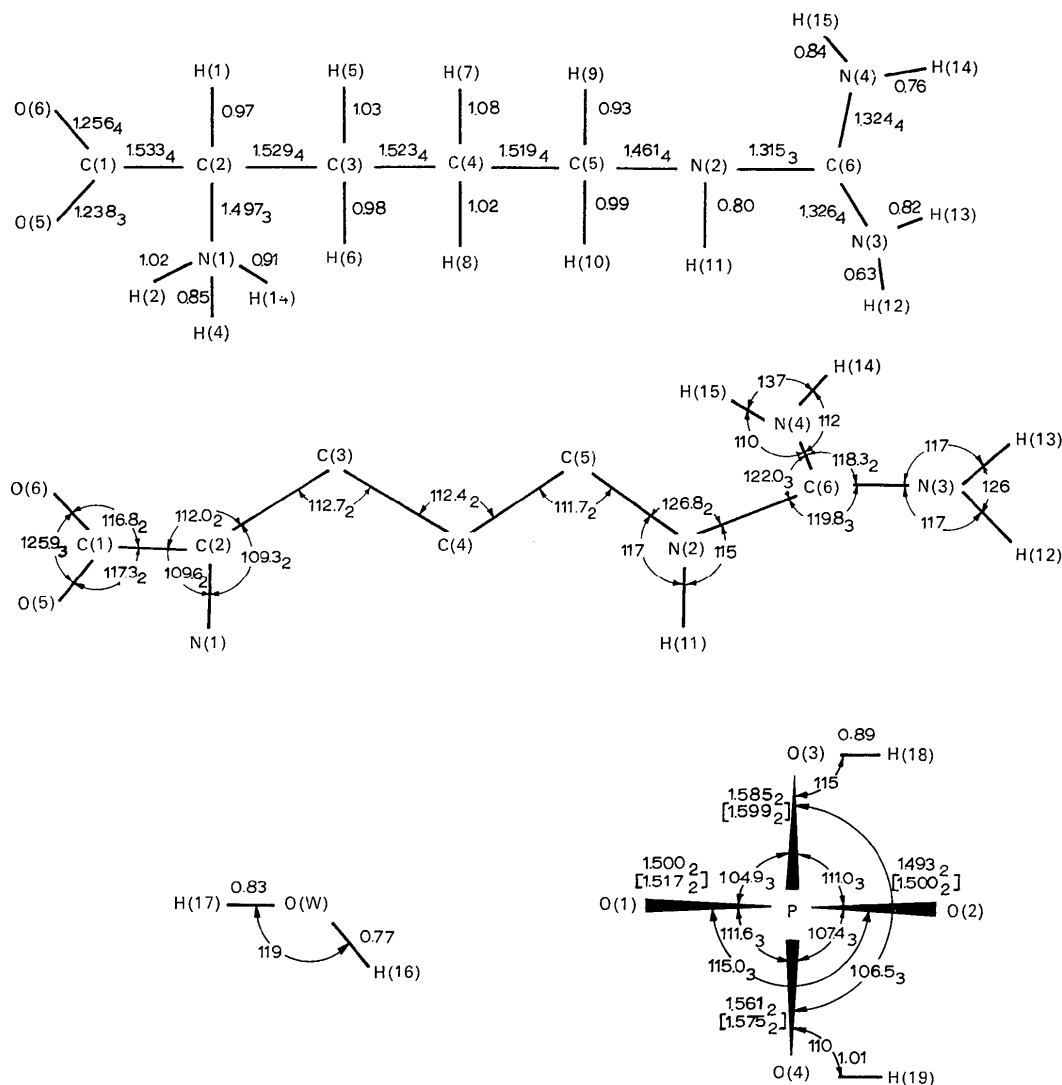


Fig. 2. Bond lengths (Å) and angles ( $^\circ$ ) in the L-arginine phosphate monohydrate structure. The subscripts indicate estimated standard deviations  $\sigma$  of the last decimal digit. The bracketed bond distances in the phosphate anion group refer to values obtained after the correction for thermal motion in which the oxygen atoms are assumed to 'ride' on the phosphorous atom (Busing, Martin & Levy, 1964). The estimated standard deviations for bond distances and angles involving hydrogen atoms are about 0.08 Å and  $5^\circ$  respectively. The angles involving protons which were not given in Fig. 2 are in the range  $104 \pm 10^\circ$ .

schematically projected onto the plane through the guanidinium group. The larger numerals indicate the interatomic  $N \cdots O$  distances (Å) while smaller numerals refer to deviations (Å) of atoms from the guanidinium plane.

The guanidinium nitrogen atoms, N(2), N(3) and N(4), are involved, as proton donors, in a number of  $N-H \cdots O$  contacts where the  $N \cdots O$  or  $H \cdots O$  distances are smaller than or equal to the sum of the corresponding van der Waals radii, 3.0 and 2.6 Å respectively (Pauling, 1962).

N(4) is hydrogen bonded to O(W), 2.901 Å, and O(3), 2.851 Å, while N(3) is involved in hydrogen bonds to O(6), 2.942 Å and O(2), 2.970 Å. The N(3)...

O(2) contact is rather unusual since the angles  $H(12)-N(3) \cdots O(2)$  and  $H(13)-N(3) \cdots O(2)$ ,  $56^\circ$  and  $72^\circ$ , exceed by far the usual angle of  $\sim 20^\circ$  (Table 3; Donohue, 1968; Fuller, 1959). A further short intermolecular contact is  $N(3) \cdots O(3)$  at 3.372 Å; since in this case the  $N(3)-H(13)$  distance of 0.82 Å is inordinately short, the  $H(13) \cdots O(3)$  distance of 2.69 Å might reduce to about 2.5 Å if an  $N(3)-H(13)$  bond length of about 1.0 Å were taken into consideration. Thus, the  $H(13) \cdots O(1)$  distance of 2.5 Å is on the verge of being called a hydrogen bonding contact.

N(2) is involved in two short contacts:  $N(2) \cdots O(W)$ , 3.104 Å and  $N(2) \cdots O(6)$ , 3.07 Å. Were the  $N(2)-H(11)$  bond assumed to be about 1.0 Å, a more realistic

Table 3. Geometrical data for short intermolecular contacts

Contacts as indicated in Figs. 3, 4 and 5.

<i>D</i>	<i>A</i>	Proton	<i>D</i> ... <i>A</i>	Distance		Angle
				<i>D</i> -H	H... <i>A</i>	H— <i>D</i> ... <i>A</i>
O[P(1)]	O(1)	H(16)	2.604 Å	0.90 Å	1.73 Å	11°
O[P(4)]	O[P(3)]	H(17)	2.551	1.01	1.56	11
O(W)	O(2)	H(18)	2.796	0.83	1.96	4
N(1)	O[P(2)]	H(10)	2.800	0.91	1.94	16
N(1)	O[P(3)]	H(8)	2.831	1.02	1.86	15
N(1)	O[P(2)]	H(9)	2.798	0.85	1.96	7
N(2)	O(1)	H(11)	3.071	0.80	2.39	28
N(2)	O(W)	H(11)	3.104	0.80	2.54	40
N(3)	O[P(1)]	H(12)	2.851	0.84	2.02	5
N(3)	O(W)	H(13)	2.901	0.75	2.20	18
N(4)	O[P(2)]	H(14)	2.970	0.82	2.84	73
N(4)	O[P(2)]	H(15)		0.63	2.68	56
N(4)	O(1)	H(15)	2.942	0.63	2.36	19
N(4)	O[P(1)]	H(14)	3.372	0.82	2.69	42

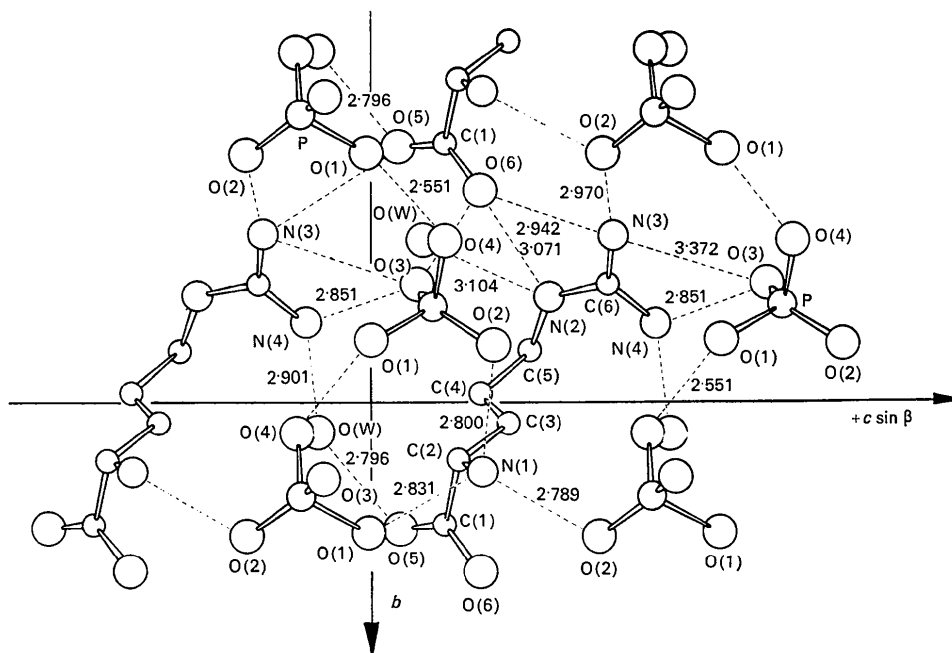


Fig. 3. Projection of the L-arginine phosphate monohydrate structure along the *a* axis. Hydrogen bonds are indicated by dashed lines.

figure than the 0.80 Å of Table 3, the  $O(W)\cdots H(11)$  and  $O(6)\cdots H(11)$  distances would be about 2.35 and 2.20 Å respectively, *i.e.* considerably less than the van der Waals  $O\cdots H$  contact of 2.6 Å. One might deduce, therefore, that the  $N(2)-H(11)$  group, being involved in two short contacts with  $O(W)$  and  $O(6)$ , forms a bifurcated hydrogen bond (Donohue, 1968; Aoki *et al.*, 1971).

The hydrogen bonds involving the carboxyl and  $N(1)$ -ammonium group, and the phosphate and water molecule are indicated from the data given in Table 3 and Figs. 3 and 4.

(d) *Packing scheme*

The molecular packing within the L-arginine phosphate monohydrate structure indicates that screw-axis related L-arginine molecules form a hydrophobic zone along the  $b$  axis which is surrounded by hydrophilic phosphate groups and water molecules (Fig. 4). The phosphate groups are arranged in sheets parallel to the  $b,c$  plane at  $a=0$  and  $a=\frac{1}{2}$ , and are hydrogen bonded to each other by an  $O(4)-H(19)\cdots O(1)$  bond and by the  $N(1)^+$  ammonium group as a mediator.

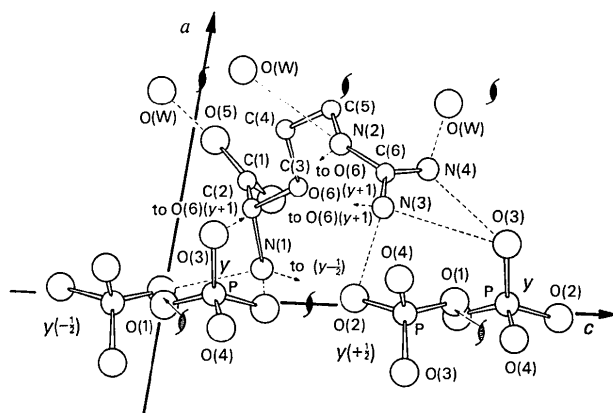


Fig. 4. As Fig. 3, but projected along the crystallographic  $b$  axis.

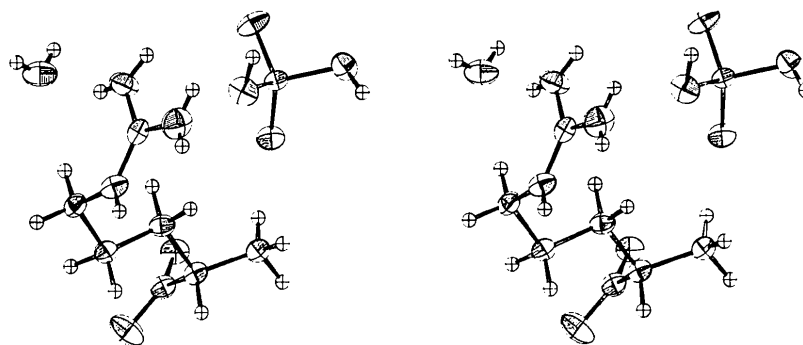


Fig. 5. Stereoscopic view along the  $b$  axis of the arginine phosphate monohydrate complex, illustrating the 50% probability thermal ellipsoids. The plot was performed using ORTEP (Johnson, 1965).

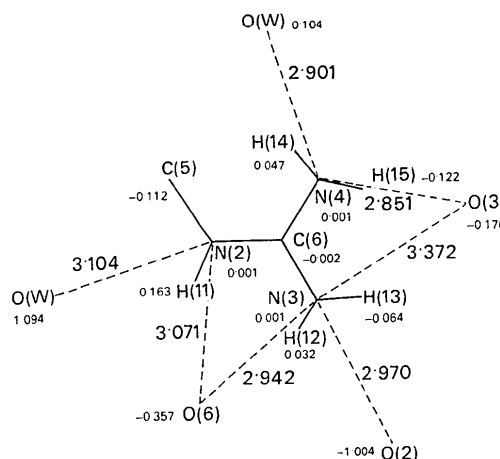
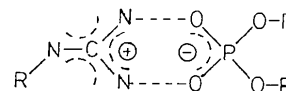


Fig. 6. A schematic projection of some atoms on the guanidinium plane, *i.e.* the mean plane through  $N(2)$ ,  $N(3)$ ,  $N(4)$  and  $C(6)$ . Large numerals indicate short intermolecular contacts while small numerals refer to deviations of the atoms from the guanidinium plane.

(e) *The L-arginine phosphate complex as a model for ionic bonds involved in basic protein-nucleic acid interactions*

It has been postulated from models and from binding studies that the ionic bond between the L-arginine side groups in basic proteins and the diester phosphate groups in DNA should be symmetrical (Suwalsky, 1968; Lewin, 1969).



A similar type of bonding was observed in the crystal structure of arginine dihydrate (Karle & Karle, 1964) involving the guanidyl and carboxyl groups, both charged, of adjacent molecules.

In the L-arginine phosphate complex, however, this type of hydrogen bonding is not realized. It is assumed that the abundance of  $N-H$  and  $O-H$  groups is

responsible for the formation of the observed intricate hydrogen bonding scheme. From this structure it is clear that the symmetrical guanidinium-phosphate interaction is by no means so favored that it is formed under all circumstances; hydrogen bonding schemes other than the proposed symmetrical arrangement are consequently also worthy of consideration in the case of basic protein-DNA interactions.

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## Molecular Conformation of the Thyroxine Analogue 3,5-Diiodo-L-thyronine N-Methylacetamide Complex (1:1)

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The crystal and molecular structure of the thyroxine analogue 3,5-diiodo-L-thyronine has been determined as a 1:1 complex with *N*-methylacetamide ( $P2_1$ ;  $Z=2$ ,  $a=7.988$ ,  $b=22.317$ ,  $c=5.995$  Å and  $\beta=95.54^\circ$ ). The structural analysis shows the planes of the two phenyl rings of the thyronine molecule to be mutually perpendicular, as expected from stereochemical interaction studies. The amino acid backbone conformation, described by the rotation about the  $C^\alpha-C^\beta$  bond, is  $300^\circ$ , showing a sterically preferred conformation. The complex is held together by a hydrogen bonding system where the amine nitrogen atom is hydrogen bonded to three oxygen atoms in a tetrahedral manner. There is also an unusually short iodine-carbonyl ( $I \cdots O=C <$ ) contact distance of 3.03 Å.

#### Introduction

Extensive studies of the molecular conformations of many amino acids and polypeptides have been made in an effort to understand structural requirements for biological activity. One such investigation has cen-

tered upon efforts to establish structure-functional requirements for the activity of thyroid hormones. (Jorgensen, 1964; Money, Kumaoka & Rawson, 1962; Barker & Shimada, 1964; Selenkow & Asper, 1955; Jorgensen & Wright, 1970). Because little crystallographic work has been done on these hormones the