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Crystallographic Studies of the Biuret Reaction. I. Potassium *bis*-Biureto Cuprate(II) Tetrahydrate, $K_2[Cu(NHCONHCONH)_2].4H_2O$*

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The crystal structure of potassium *bis*-biureto cuprate(II) tetrahydrate, $K_2[Cu(NHCONHCONH)_2]$. $4H_2O$, has been determind, using the 3-dimensional X-ray diffraction intensities obtained with CuKα radiation. Hydrogen atoms were located, and individual anisotropic thermal parameters were included in the refinement.

The copper atom in this complex is square-coordinated by four amide nitrogen atoms belonging to two (biuret)$^{=}$-residues. The usual elongated octahedron about the copper atom is completed by two more distant nitrogen atoms, each of which belongs to one of the biuret residues in one of two neighbouring complexes.

The Cu–N–C angles at the two indepedent kinds of ligand nitrogen atoms are 131°. The configurations about these atoms seem to be distortedly trigonal. The complexes are not entirely planar, though the known dimensions of the biuret molecule are not altered significantly by coordination. The notwork of hydrogen-bonds is not particularly efficient.

The final R factor is 0·079 (including the unobserved reflections) and the standard deviations of the coordinates of the light atoms are about 0·007 Å.

1. Introduction

This investigation arose partly out of a general interest in metal–peptide inter-action (Freeman, Smith & Taylor, 1959) and partly out of a specific interest in the 'biuret reaction'.

It has been known for a long time that, when cupric ion is added to an alkaline solution of a protein or polypeptide, a violet-red colour is produced. This colour is reversibly changed to blue upon acidification of the solution. The reaction is a general one, but one of the simplest substances which undergo it is biuret, $NH_2CONHCONH_2$, (Brücke, 1883; Schiff, 1896) and the reaction has become identified with the name of this compound.

It was realized early that the violet-red colour was associated with the formation of a metal–peptide complex. Electrolysis of a copper–biuret solution showed the complex to be anionic (Ley & Werner, 1913; Rising, Hicks & Moerke, 1930), and Kober & Haw (1916; cf. Plekhan, 1952) ascribed the violet-red colour ($\lambda_{\max.} \approx 505\,m\mu$) to the presence of four nitrogen atoms about the copper. No structural studies on the nature of the biuret-reaction complexes have, however, been reported.

2. Data

Potassium *bis*-biureto cuprate(II) tetrahydrate was prepared by shaking a fresh concentrated solution of cupric acetate (0·02 mole) with biuret (0·04 mole) dissolved in the calculated excess of aqueous potassium hydroxide (0·1 mole in 80 ml.). Violet-red crystals were obtained in 1–2 days after the addition of much alcohol (2 l.) containing a little alkali (Schiff, 1896, 1898). The crystals were unstable in air, but were adequately protected during the X-ray exposures by a thin film of collodion.

The space group was identified from systematic absences of reflections on Weissenberg photographs. The lattice constants were determined by fitting them to the spacings of six planes which gave reflections at large angles on Weissenberg photographs. These were taken with Cu $K\alpha$ radiation on a large-radius camera and were calibrated by the superposition of the powder lines from a platinum wire on each film. An independent and identical value for d_{100} was obtained from a rotation photograph using the camera-adapter and extrapolation procedure of Mathieson (1957).

$C_4H_{14}O_8N_6CuK_2$, $M=376{\cdot}8$ (Cu $K\alpha$, $\lambda=1{\cdot}5418$ Å).

Monoclinic,

$$a=3{\cdot}843\pm0{\cdot}004,\ b=13{\cdot}321\pm0{\cdot}008,$$
$$c=13{\cdot}916\pm0{\cdot}008\ \text{Å};\ \beta=94^\circ\ 5'\pm15'.$$

$U=710{\cdot}5$ Å^3. For $Z=2$, $D_c=1{\cdot}761$ g.cm.$^{-3}$. D_m not observed. $F(000)=414$.

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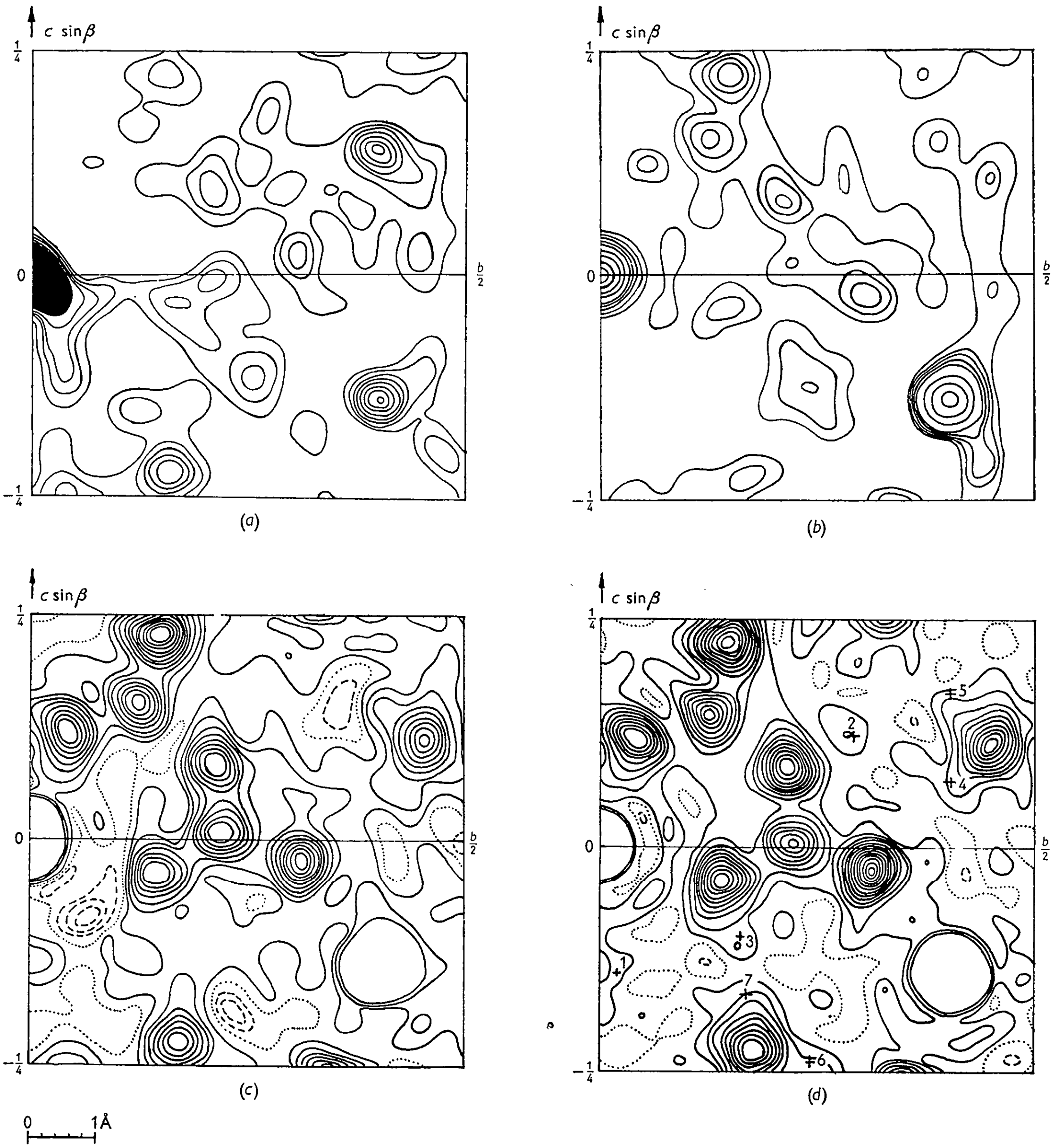

Fig. 1. Development of *a*-axis projection of $K_2Cu(NHCONHCONH)_2.4\,H_2O$. (*a*) Scale arbitrary. (*b*) Scale arbitrary. After fourth contour, only one contour in four is shown. (*c*), (*d*) Contours at 1 e.Å^{-2}, negative contours dashed, zero contour dotted. Crosses indicate probable hydrogen positions.

Systematic absences:

*h*0*l* absent for (*h*+*l*) odd,
0*k*0 absent for *k* odd.

Space-group: $P2_1/n\text{–}C_{2h}^5$.

The intensities of 929 0*kl*, 1*kl* and *hkL* (*L*=0 to 8) reflections were estimated visually on Weissenberg photographs. In addition, 331 reflections in the same range were recorded as unobservably weak, and 38 of the $\bar{3}kl$, $\bar{3}k2$, $\bar{4}kl$ and $\bar{4}k2$ reflections were missed through an error in the setting of the Weissenberg camera oscillation. Corrections to the observations were applied for the Lorentz, polarization and Tunell factors. The linear absorption coefficient of the crystals for Cu *Kα* radiation was 80 cm.$^{-1}$. The 0*kl* and 1*kl* data did not require absorption corrections since the crystal (0·3 × 0·04 × 0·04 mm.^{3}) used for these observations was rotated about its needle axis. For the *hk*0 ... *hk*8 photographs, another crystal (0·3 × 0·1 × 0·1 mm.^{3}) was rotated about an axis perpendicular to its long axis. In these cases the transmission factor varied from 10 to 45% and absorption was thus a serious problem.

Absorption corrections for the c-axis data were calculated *via* the method of Joel, Vera & Garaycochea (1953). Contours of these corrections, calculated at 40 values of (α, θ) per quadrant in a reciprocal lattice layer—where α is the Weissenberg angular coordinate and θ is the Bragg angle—were plotted on templates. The correction for each reflection could then be read to an estimated accuracy of 3% (of the calculable value) by placing the templates over the Weissenberg films.

An approximate scale factor for the $0kl$ data was obtained by Wilson's (1942) method. The other data were brought to the same scale *via* groups of common reflections. From a comparison of the final scaled F^2 values of reflections observed about both axes it was concluded that the relative standard deviations of the intensities were about 20% for low intensities, 10% for medium intensities ($100 < F^2 < 1600$) and 5% for strong reflections.

3. Structure determination

Approximate structure

The structure was solved in the projection along the short a-axis. Since the copper atom had to lie in a position of two-fold symmetry, an $0kl$ Fourier projection was calculated with all signs positive (Fig. 1(a)). This showed the location of the potassium ion. Successive projections showed, in turn, the biuret molecule (Fig. 1(b)) and the two water molecules (Fig. 1(c)) in the asymmetric unit. One further projection indicated small coordinate shifts, and the next corresponded to complete phase-determination in this zone (Fig. 1(d)).

One of the prime objects of this work was to discover whether the copper was bound to the biuret molecule at the amide nitrogen or oxygen atoms. In the structure-factor calculations associated with the above electron-density projections, all the atoms of the biuret molecule were initially given equal weights as nitrogen atoms. In the electron-density maps, however, the atoms of the amide groups from their first appearance had peak heights which left little doubt that nitrogen atoms were bonded to the central copper atom. The $0kl$ structure-factors calculated for this configuration of the ligand molecule, using positional coordinates read from the last Fourier projection with a temperature-factor $\exp(-2{\cdot}2 \sin^2\theta/\lambda^2)$, had a reliability-factor (for the observed reflections) $R = \Sigma|F_o - F_c|/\Sigma|F_o| = 0{\cdot}122$. If either amide group was inverted so that it was coordinated to the metal *via* an oxygen atom, the agreement between the F_o's and F_c's became worse by about 1%.

An $0kl$-$(F_o - F_c)$-synthesis now not only indicated the expected changes in some coordinates and in the weights or temperature-parameters of the heavier atoms, but also had peaks corresponding to seven hydrogen atoms (Fig. 2). These provided strong evidence that the structure contained K^+ and $[Cu(NHCONHCONH)_2]^=$ ions and H_2O molecules instead of, e.g., K^+ and OH^- ions and $[Cu(NH_2CONHCONH_2)_2]^{++}$ complexes. While the important, tentative conclusions regarding the nature of the complex ion and the identities of the coordinating atoms were thus reached at an early stage of the work, they were substantiated by everything that happened in the further refinement of the structure.

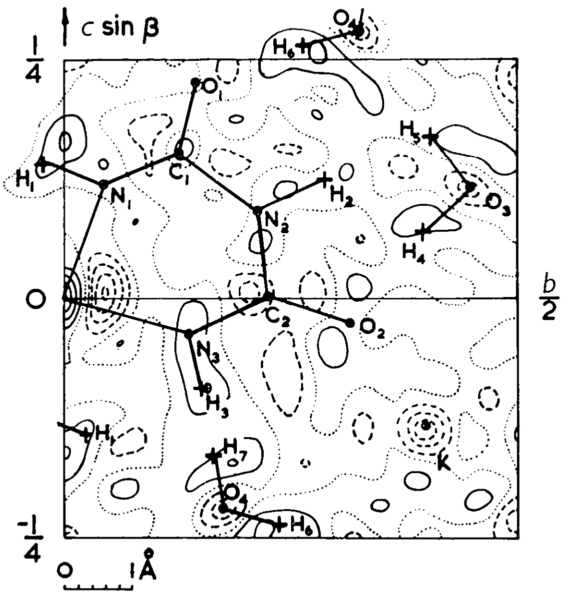

Fig. 2. $0kl(F_o - F_c)$ projection. Contours at 0·5 e.Å^{-2}, negative contours dashed, zero contour dotted. Atomic positions from projection shown in Fig. 1(d), hydrogen atoms omitted from F_c.

The high intensity of the $\bar{1}03$ reflection ($|F_o| = 184$) made it likely that the chelating molecules lay in this plane. The angle between the ($\bar{1}03$) and (100) planes was 38°. The bond-lengths of the amide-groups in the $0kl$-projection were compatible with those of normal amide groups tilted at this angle from the (100) plane. Approximate x-coordinates for the atoms of the biuret residues were calculated on this assumption. The potassium ion was placed at $x = 0{\cdot}5$ on the basis of a strong peak at (0·50, 0·80) on an $hk0$ Patterson projection, and of the reasonableness of the resultant $K^+ \cdots O$ contacts with the oxygen atoms of the nearest biuret residues.

These x-coordinates were adequate for attaching signs to the 320 observed $F(1kl)$'s, whose scale at this stage was only approximate. Generalized projections $C_1(yz)$ and $S_1(yz)$ were computed from the first-layer data, and as a result the x-coordinates of the two H_2O molecules could also be approximately determined. When the H_2O molecules were included in the calculated $F(1kl)$'s, R fell from 0·33 to 0·29.

Further refinement of the combined zero- and first-layer a-axis data by generalized projections and least-squares quickly gave much improved agreement, the $F(1kl)$'s being scaled to their calculated values.

The x-coordinates of some atoms, however, converged on values which corresponded to non-planar amide-groups with unlikely bond-lengths. These anomalous structural features disappeared when comparison with the (by now) absorption-corrected c-axis data led to a 10% revision of the scale of the $1kl$ intensities.

Refinement of coordinates

A least-squares refinement program for the Sydney University computer SILLIAC (Freeman, 1958) permitted the initial adjustments of the positional parameters to be carried out rapidly. While this program had provision for only one over-all two-parameter temperature factor $\exp(-B\sin^2\theta/\lambda^2-\alpha h^2)$, it had the virtues of availability and very high speed. The $0kl$, $1kl$ and $hk0$ data were accordingly submitted to three cycles of refinement, which reduced R for these reflections from 0·147 to 0·113. The refinement process was now extended to the complete data. After five least-squares cycles, the over-all reliability factor R had dropped from 0·144 to 0·124. When the hydrogen atoms were included (see below), R was 0·120. The final temperature-parameters were $B=2·24$ and $\alpha=0·01$. The greatest coordinate change in the last cycle was 0·003 Å in the x-direction, and the standard deviations of the positional parameters of the light atoms were between 0·02 and 0·03 Å.

The weighting system throughout this part of the refinement was that of Hughes (1941), i.e.

$$\begin{aligned}
&\sqrt{w}=1/|F_o| \text{ for } |F_o| \geq 4|F_{\text{min.}}|,\\
&\sqrt{w}=\tfrac{1}{4}|F_{\text{min.}}| \text{ for } |F_o|<4|F_{\text{min.}}| \text{ and for}\\
&\qquad |F_c|>|F_{\text{unobs.}}|, \text{ and}\\
&\sqrt{w}=0 \text{ for } |F_c| \leqq |F_{\text{unobs.}}|.
\end{aligned}$$

($|F_{\text{min.}}|$ was the smallest observed F, and $|F_{\text{unobs.}}|$ was the maximum value of an unobserved reflection.) With these weights, $\Sigma w(\Delta F)^2$ was 50·5.

The parameters from the last SILLIAC least-squares cycle were supplemented by the calculation of individual isotropic temperature factors for all atoms except the hydrogens. For this purpose, Cochran's method (1951) was applied to an $0kl$ (F_o-F_c)-projection. This step probably did not reduce the later computation. In view of the extent to which the refinement of the anisotropic thermal parameters was ultimately pushed, a single isotropic temperature parameter would have been just as adequate a starting point. Seen in retrospect it would also have been a more correct early strategy, once the structure was established, to use all the available data instead of seeking economy by using only a limited number of F's at the start.

The further refinement of the structure was carried out on a Datatron 205 computer. The program used only the diagonal terms of the least-squares matrix to evaluate the coordinate shifts, but for each atom a set of seven seven-term normal equations was solved to derive a scaling-factor and six coefficients for the anisotropic temperature factor

$$\exp(-4\beta_i'\sin^2\theta)\exp\tfrac{1}{10}(\alpha_i'h^2+\gamma_i'l^2+\delta_i'hk+\varepsilon_i'kl+\eta_i'hl)\,.$$

The average of the scale factors for all atoms was substituted in all of the sets of normal equations which were then re-solved for the thermal parameters. [The final values of these parameters were converted to the parameters for the standard form of the temperature factor,

$$\exp\,-(\alpha_i h^2+\beta_i k^2+\gamma_i l^2+\delta_i hk+\varepsilon_i kl+\eta_i lh)\,.$$

In this form they have been listed in Table 2.]

In this manner, seven refinement cycles were carried out. The weights attached to the F's were the same as before, with the exception that all unobservably small reflections were given $\sqrt{w}=0$ for the first six cycles. The shifts indicated by an eighth cycle were not applied to the parameters; this cycle was used only to produce the final lists of structure-factors and the estimates of the various standard deviations.

The hydrogen atoms were included in the calculated structure-factors, but their coordinates and their common isotropic temperature factor were not automatically adjusted. After five cycles of refinement, the hydrogen positions were reviewed in the light of the changes which had occurred in the coordinates of the atoms to which they were presumed to be attached. This led to the discovery and correction of an arithmetical error in the original calculation of five of the hydrogen coordinates. As a result of this dis-

Table 1. *Final positional parameters, their standard deviations σ, and final thermal parameters for atomic temperature-factors*

$$T_i=\exp\,-(\alpha_i h^2+\beta_i k^2+\gamma_i l^2+\delta_i hk+\varepsilon_i kl+\eta_i hl)$$

Atom	x_i	y_i	z_i	$10^4\sigma(x_i)$	$10^4\sigma(y_i)$	$10^4\sigma(z_i)$	$10^4\alpha_i$	$10^4\beta_i$	$10^4\gamma_i$	$10^4\delta_i$	$10^4\varepsilon_i$	$10^4\eta_i$
C_1	0·3882	0·1301	0·1469	17	5	6	384	27	21	26	−6	81
C_2	0·0968	0·2226	0·0021	16	4	6	316	23	27	−37	11	69
N_1	0·2800	0·0396	0·1146	16	4	5	535	23	31	−14	13	−80
N_2	0·3034	0·2123	0·0893	15	4	5	467	21	32	12	1	−37
N_3	−0·0251	0·1386	−0·0408	15	4	5	464	25	22	−9	−9	−3
O_1	0·5557	0·1477	0·2251	14	4	4	591	33	19	−5	−25	−127
O_2	0·0491	0·3125	−0·0272	14	4	4	685	23	20	26	5	−65
O_3	0·9379	0·4469	0·1160	19	4	5	832	35	38	−32	5	56
O_4	0·8621	0·3259	0·2786	17	5	5	716	50	43	57	−7	107
K^+	0·5503	0·4030	−0·1387	4	1	1	417	35	26	18	3	−28
Cu	0·0000	0·0000	0·0000	—	—	—	458	20	18	12	−2	−52

turbance, the average shifts indicated by the sixth least-squares cycle were as large as had been those in the fifth. After this, convergence was normal.

At the time of the correction of the hydrogen parameters the opportunity was taken of making a critical comparison of all the F_o and F_c. In seven cases the disagreements were unusually large, and had shown no tendency to become smaller during the refinement. The original films were re-examined by one of the authors who had no knowledge of the directions of the disagreements. For three reflections this procedure led to the amendment of what had been incorrect estimates; for three others the gap was narrowed in the next two cycles; but for $F(\bar{1}03)$, the large discrepancy was not reduced and was ascribed to extinction.

The average standard deviations of the atomic coordinates calculated after the seventh and eighth cycles were equal. The refinement was therefore stopped. The final reliability factor (including unobservably small reflections) was $R=0{\cdot}079$, and $\Sigma w(\Delta F)^2$ had decreased to 14·45.

The coordinates, except those of the hydrogen atoms, are listed with their standard deviations in

Table 2. *Observed and calculated structure factors for potassium* bis-*biureto cuprate*(II) *tetrahydrate*

Results in each group are in order of increasing k. Each line shows k, $10\lvert F_o\rvert$ and $10F_c$

k	$10\lvert F_o\rvert$	$10F_c$
0,k,0		
2	217	203
4	253	-229
6	403	358
8	181	201
10	364	333
12	98	100
14	127	113
16	<70	-11
0,k,1		
2	595	-594
3	259	250
4	248	-221
5	804	801
6	339	307
7	637	61.
8	292	252
9	377	345
10	181	-144
11	192	-180
12	83	-86
13	290	267
14	96	-79
15	197	209
16	<70	-30
17	71	62
0,k,2		
0	592	616
1	83	46
2	236	236
3	250	247
4	791	804
5	443	-403
6	300	274
7	466	-438
8	248	229
9	386	-334
10	362	327
11	<56	38
12	<57	47
13	321	320
14	192	192
15	<42	49
16	133	137
0,k,3		
1	718	708
2	279	-256
3	651	649
4	173	152
5	239	-207
6	313	-281
7	<75	29
8	364	314
9	550	569
10	<68	41
11	282	265
12	254	-251
13	264	253
14	<57	-51
15	<68	8
16	<65	57
0,k,4		
0	<55	-28
1	<45	-6
2	476	-429
3	<55	5
4	501	523
5	404	-370
6	992	978
7	84	45
8	272	273
9	216	181
10	<100	74
11	84	-71
12	238	234
13	109	-91
14	242	259
15	<40	-11
16	194	207
0,k,5		
1	690	722
2	536	535
3	209	201
4	375	342
5	75	103
6	447	-396
7	382	355
8	196	-201
9	242	215
10	<57	51
11	209	190
12	133	128
13	271	277
14	85	94
15	<50	64
16	<50	-27
0,k,6		
0	439	480
1	267	229
2	422	414
3	214	-204
4	201	175
5	<52	42
6	133	108
7	544	516
8	360	343
9	171	166
10	418	383
11	<53	-18
12	213	223
13	188	-198
14	<53	67
15	145	-133
16	100	100
0,k,7		
1	151	-134
2	142	128
3	688	713
4	82	82
5	517	497
6	183	-176
7	379	342
8	<52	6
9	217	211
10	145	165
11	<100	-73
12	<45	-55
13	114	128
14	<37	54
15	188	209
0,k,8		
0	916	981
1	432	422
2	493	478
3	267	238
4	220	185
5	<72	20
6	<62	17
7	<95	-20
8	196	175
9	82	-74
10	254	244
11	<95	84
12	140	127
13	<85	10
14	<70	37
15	<33	58
0,k,9		
1	359	350
2	362	-388
3	118	106
4	154	-145
5	417	419
6	183	161
7	216	211
8	202	182
9	205	189
10	<100	82
11	127	134
12	196	-192
13	144	145
14	<60	-55
0,k,10		
0	149	109
1	232	222
2	124	159
3	<90	-6
4	577	622
5	92	-99
6	304	310
7	116	-104
8	<100	24
9	265	-254
10	<95	42
11	<85	109
12	75	60
13	125	133
14	132	135
0,k,11		
1	306	334
2	<95	61
3	286	302
4	<100	99
5	117	102
6	129	-115
7	175	-163
8	<95	-35
9	371	378
10	<90	42
11	298	291
12	82	-51
13	84	78
0,k,12		
0	<95	-56
1	<95	-46
2	<95	65
3	161	-172
4	340	382
5	212	-201
6	150	164
7	92	102
8	194	190
9	133	123
10	159	161
11	<70	-46
12	75	57
0,k,13		
1	181	202
2	166	152
3	<95	39
4	222	215
5	291	305
6	210	-185
7	123	110
8	<85	-107
9	130	97
10	<70	-4
0,k,14		
0	324	329
1	<95	-72
2	160	137
3	91	-44
4	<95	-13
5	<90	66
6	<90	6
7	146	141
8	200	196
9	<70	-26
10	206	218
0,k,15		
1	<90	-18
2	200	-198
3	191	190
4	<81	18
5	259	308
6	<90	71
7	92	83
0,k,16		
1	<81	68
2	209	219
3	178	170
4	186	191
5	<70	68
0,k,17		
1	176	178
2	115	-121
3	<66	70
4	<66	-45
5	73	79
1,k,0		
1	375	377
2	535	-545
3	542	579
4	119	-96
5	271	-232
6	286	-269
7	211	186
8	134	125
9	644	678
10	<61	-84
11	345	341
12	<67	-14
13	240	239
14	<57	-8
15	<58	-25
16	<70	49
1,k,1		
0	435	489
1	371	-375
2	<36	16
3	109	-93
4	551	560
5	422	-393
6	739	790
7	<54	-3
8	<57	59
9	299	268
10	64	-32
11	166	-135
12	180	169
13	112	-107
14	212	210
15	<43	32
16	246	251
1,k,2		
1	360	363
2	876	984
3	<41	14
4	414	420
5	103	126
6	284	-287
7	609	631
8	118	-126
9	254	270
10	61	-57
11	199	197
12	129	123
13	324	350
14	<48	45
15	80	88
16	<60	-34
1,k,3		
0	881	886
1	<31	-12
2	281	312
3	417	-445
4	80	-64
5	219	-223
6	220	219
7	302	299
8	359	372
9	157	148
10	417	415
11	<68	37
12	248	268
13	107	-103
14	86	90
15	75	-65
16	86	102
1,k,4		
1	75	-30
2	191	204
3	939	971
4	224	229
5	398	429
6	115	103
7	393	370
8	132	105
9	166	176
10	124	113
11	186	-173
12	<62	-57
13	172	172
14	58	-69
15	244	256
16	<36	25
1,k,5		
0	859	876
1	434	445
2	248	243
3	439	436
4	213	213
5	45	18
6	172	152
7	139	-123
8	298	298
9	57	-39
10	420	435
11	70	113
12	181	162
13	47	26
14	41	48
15	44	73
16	36	50
1,k,6		
1	693	739
2	365	-397
3	245	249
4	316	-303
5	308	311
6	55	47
7	222	211
8	311	294
9	175	185
10	101	108
11	<50	37
12	204	-192
13	163	159
14	<41	-44
15	<28	35
1,k,7		
0	356	370
1	235	269
2	<52	27
3	163	161
4	592	656
5	<52	8
6	564	574
7	55	52
8	57	21
9	202	-202
10	<53	16
11	<45	31
12	<45	42
13	63	79
14	160	176
15	<22	-33
1,k,8		
1	338	375
2	<52	-13
3	403	392
4	<53	59
5	<52	72
6	310	-292
7	<85	-88
8	130	-123
9	482	472
10	<70	5
11	299	297
12	103	61
13	116	134
14	<60	42
1,k,9		
0	141	125
1	122	-96
2	214	218
3	202	-180
4	525	545
5	275	-254
6	155	143
7	193	162
8	80	78
9	183	167
10	214	204
11	<95	-76
12	<81	51
13	154	-153
14	116	117
1,k,10		
1	<95	37
2	250	254
3	162	143
4	305	297
5	373	374
6	364	-338
7	232	224
8	<95	-43
9	205	193
10	<95	5
11	113	91
12	<56	-5
13	153	164
1,k,11		
0	536	575
1	<95	-21
2	213	198
3	82	-40
4	113	-67
5	149	-112
6	97	52
7	<95	93
8	218	205
9	<89	-18
10	272	275
11	<79	-5
12	196	214
1,k,12		
1	<95	10
2	257	-259
3	263	254
4	136	119
5	307	322
6	152	129
7	164	168
8	190	157
9	<79	51
10	<81	-26
1,k,13		
1	111	97
2	250	223
3	204	199
4	253	276
5	<95	61
6	<92	47
7	<89	-62
8	153	124
9	160	-146
10	135	161
1,k,14		
2	168	-143
3	225	210
4	<89	-4
5	<84	49
6	<79	11
7	<74	-10
8	146	128
9	134	133
1,k,15		
6	278	258
2,k,0		
0	835	899
1	<47	-25
2	361	347
3	<50	-45
4	171	-169
5	<63	-3
6	160	131
7	224	210
8	236	281
9	172	142
10	259	270
11	<57	-25
12	199	223
13	116	-123
14	91	88
15	50	-61
2,k,1		
1	236	-234
2	<44	-8
3	465	483
4	<52	-16
5	387	371
6	145	154
7	468	504
8	69	75
9	188	226
10	68	39
11	<62	-88
12	<57	36
13	169	196
14	81	-98
15	233	238
2,k,2		
0	846	849
1	170	151
2	370	357
3	378	377
4	194	215
5	<50	50
6	109	95
7	244	-239
8	168	169
9	<62	-46
10	277	281
11	115	128
12	81	85
13	70	71
14	58	70
15	71	76
2,k,3		
1	592	597
2	126	-86
3	185	184
4	141	-127
5	156	164
6	178	188
7	211	231
8	388	373
9	239	274
10	<71	45
11	109	91
12	185	-181
13	120	136
14	91	-113
15	<29	27
2,k,4		
0	362	392
1	69	79
2	70	-39
3	69	55
4	498	520
5	175	-171
6	600	649
7	<66	-8
8	<68	60
9	94	-64
10	<61	-10
11	<57	-2
12	<51	17
13	80	91
14	159	183
15	<21	34
2,k,5		
1	539	567
2	129	132
3	391	418
4	146	140
5	<53	8
6	212	-188
7	<62	-28
8	81	-92
9	298	312
10	57	-75
11	165	156
12	111	112
13	114	139
14	<29	21
2,k,6		
0	72	87
1	249	-247
2	125	133
3	178	-167
4	439	437
5	192	-177
6	184	190
7	168	140
8	142	123
9	185	179
10	276	293
11	81	-85
12	97	99
13	136	-149
14	83	69
2,k,7		
1	<42	13
2	219	209
3	327	354
4	161	150
5	335	345
6	459	-457
7	231	210
8	<61	-39
9	135	121
10	<51	3
11	<40	32
12	<37	27
13	132	149
14	42	73
2,k,8		
0	589	647
1	<66	85
2	201	201
3	207	158
4	57	-108
5	108	-131
6	94	96
7	<89	70
8	156	177
9	<86	49
10	354	304
11	<62	-51
12	213	227
13	<36	16
3,k,0		
1	407	381
2	172	-152
3	65	89
4	219	-182
5	77	50
6	<72	85
7	148	142
8	217	219
9	202	222
10	<70	12
11	151	127
12	37	-46
13	68	70

Table 2 (*cont.*)

3,k,1

0	262	274
1		
2	92	84
3	132	119
4	356	348
5	<62	-38
6	397	424
7	<62	56
8	<57	28
9	<62	31
10	<65	-77
11	<56	-63
12	<62	-27
13	<57	22

3,k,2

1	325	333
2	179	183
3	168	173
4	118	97
5	59	21
6	121	-115
7	92	113
8	66	-62
9	210	224
10	99	-100
11	140	132
12	116	137
13	120	115

3,k,3

0	216	234
1	316	-311
2	195	214
3	177	-186
4	209	232
5	131	-130
6	133	116
7	<74	62
8	77	69
9	147	148
10	152	158
11	<52	-61
12	65	68
13	55	-89

3,k,4

1	65	-42
2	200	196
3	318	322
4	146	146
5	265	275
6	263	-245
7	211	204
8	<65	-6
9	60	42
10	<51	-32
11	<41	13
12	<33	24

3,k,5

0	407	427
1	54	58
2	161	161
3	176	146
4	123	-105
5	201	-205
6	63	83
7	<61	-77
8	168	161
9	55	71
10	315	280
11	<36	-28
12	166	201

3,k,6

1	111	100
2	162	-150
3	176	186
4	120	-115
5	102	104
6	<61	35
7	306	288
8	241	234
9	<52	49
10	<43	-22
11	<32	23
12	<17	-71

3,k,7

0	51	-36
1	70	74
2	128	123
3	203	217
4	180	192
5	99	103
6	209	214
7	<54	-63
8	101	101
9	118	-119
10	74	86
11	<23	67

3,k,8

1	226	208
2	58	-86
3	271	279
4	77	24
5	<79	-96
6	<79	26
7	<77	12
8	<71	55
9	219	202
10	<45	58

4,k,0

0	150	144
1	184	-179
2	149	163
3	67	-76
4	68	65
5	<67	-20
6	83	67
7	60	75
8	87	80
9	101	101
10	68	78

4,k,1

1	59	-53
2	83	55
3	182	184
4	<62	32
5	171	191
6	128	-138
7	176	170
8	48	-22
9	<38	-19
10	<67	-23

4,k,2

0	256	260
1	<50	44
2	165	180
3	142	144
4	51	-69
5	104	-108
6	55	17
7	71	-101
8	60	83
9	<67	40

4,k,3

1	<57	56
2	63	-60
3	63	77
4	127	-131
5	<62	34
6	<58	28
7	235	230
8	193	180
9	<30	52

4,k,4

0	<58	-19
1	<43	43
2	79	87
3	119	115
4	88	92
5	53	23
6	168	187
7	75	-79
8	117	87
9	<30	-63

4,k,5

1	202	181
2	<52	-4
3	156	151
4	<50	33
5	95	-105
6	42	69
7	<38	41
8	<28	11

4,k,6

0	69	-65
1	<50	-32
2	49	72
3	57	-76
4	223	210
5	<42	-1
6	176	171
7	115	104

4,k,7

1	75	94
2	235	220
3	184	167
4	38	48
5	119	105
6	92	-82

4,k,8

0	282	271
1	<58	-6
2	102	123
3	<54	-50
4	<48	14
5	<36	-39

$\bar{1}$,k,1

0	818	748
1	257	258
2	392	382
3	660	-670
4	724	670
5	242	-210
6	430	398
7	309	292
8	222	224
9	337	310
10	163	142
11	<68	34
12	<72	-6
13	268	-264
14	203	201
15	<54	41
16	177	161

$\bar{1}$,k,2

1	854	839
2	711	747
3	193	200
4	653	584
5	635	569
6	156	-140
7	530	507
8	291	-277
9	178	194
10	180	175
11	189	-201
12	<70	-39
13	216	212
14	46	64
15	182	192
16	<48	1

$\bar{1}$,k,3

0	1841	2186
1	109	98
2	<49	-27
3	444	-424
4	141	-145
5	102	-84
6	351	333
7	64	66
8	361	362
9	129	-104
10	428	433
11	<65	46
12	160	162
13	<60	-12
14	112	95
15	<44	7
16	<28	-46

$\bar{1}$,k,4

1	412	372
2	459	-479
3	517	515
4	288	-279
5	672	707
6	<52	36
7	202	223
8	121	103
9	150	134
10	<61	-16
11	165	-168
12	114	-116
13	159	166
14	<47	9
15	193	208
16	<47	11

$\bar{1}$,k,5

0	133	154
1	525	520
2	145	155
3	432	465
4	538	532
5	163	-145
6	134	119
7	178	-186
8	278	283
9	229	-215
10	352	368
11	<57	-18
12	123	117
13	152	147
14	168	168
15	<35	19
16	<16	61

$\bar{1}$,k,6

1	426	421
2	333	-358
3	204	238
4	147	-150
5	59	53
6	365	-351
7	88	92
8	227	212
9	392	431
10	139	141
11	275	280
12	213	-230
13	203	212
14	<39	10
15	<60	14

$\bar{1}$,k,7

0	124	130
1	133	150
2	<70	30
3	118	115
4	461	495
5	55	-58
6	543	578
7	77	93
8	132	111
9	112	84
10	<53	-70
11	<51	-14
12	140	138
13	88	-82
14	209	219
15	63	-63

$\bar{1}$,k,8

1	270	311
2	310	337
3	67	79
4	278	282
5	135	117
6	207	-189
7	211	206
8	129	-140
9	315	308
10	<72	69
11	295	308
12	<60	60
13	126	164
14	41	54

$\bar{1}$,k,9

0	223	225
1	126	97
2	439	453
3	280	-291
4	265	261
5	140	-127
6	<95	-17
7	272	290
8	212	213
9	158	151
10	188	175
11	<85	1
12	104	102
13	140	-127

$\bar{1}$,k,10

1	<90	8
2	202	193
3	349	374
4	179	189
5	368	389
6	<95	-4
7	220	208
8	<100	-8
9	<95	90
10	<90	92
11	<81	-34
12	98	-86

$\bar{1}$,k,11

0	434	473
1	149	135
2	249	274
3	<95	-24
4	<95	65
5	<95	-1
6	<95	4
7	<95	-75
8	204	207
9	<90	-101
10	181	208
11	<90	77
12	127	153

$\bar{1}$,k,12

1	230	244
2	263	-277
3	169	157
4	159	-129
5	315	360
6	183	172
7	<95	38
8	<90	57
9	<85	13
10	<85	46
11	99	92
12	107	-102

$\bar{1}$,k,13

0	<95	-117
1	185	188
2	<100	84
3	157	75
4	347	398
5	<95	10
6	128	140
7	<81	-92
8	<81	82
9	159	-166
10	104	99

$\bar{1}$,k,14

1	202	232
2	<95	13
3	147	139
4	<90	-82
5	<81	62
6	81	-87
7	110	-110
8	<65	-31
9	231	251

$\bar{1}$,k,15

0	<90	4
1	<84	-26
2	<85	48
3	<85	-64
4	221	225
5	<70	-14
6	116	113
7	<70	74
8	110	126

$\bar{1}$,k,16

1	105	109
2	<81	76
3	<70	-48
4	110	111
5	175	185
6	<70	-46
7	69	88

$\bar{1}$,k,17

0	212	233
1	83	-99
2	102	121
3	105	-88

$\bar{2}$,k,1

1	227	257
2	488	-522
3	116	110
4	<57	44
5	209	176
6	370	353
7	476	429
8	179	156
9	146	105
10	142	84
11	200	147
12	222	-195
13	265	251
14	91	-91
15	103	97

$\bar{2}$,k,2

3	<62	44
4	429	421
5	49	48
6	571	524
7	61	-71
8	183	170
9	264	-207
10	64	58
11	181	149
12	182	166
13	114	113
14	148	126
15	<57	-4

$\bar{2}$,k,3

1	601	659
2	306	319
3	415	462
4	<53	-8
5	169	-179
6	<65	37
7	<70	40
8	<77	7
9	346	326
10	197	164
11	252	214
12	138	-107
13	126	112
14	<42	13

$\bar{2}$,k,4

0	188	183
1	288	293
2	161	191
3	240	-249
4	328	336
5	<52	-50
6	405	383
7	236	240
8	302	289
9	220	211
10	<65	36
11	<62	0
12	<55	17
13	243	-260
14	165	175
15	<52	17

$\bar{2}$,k,5

1	485	497
2	217	242
3	73	67
4	159	147
5	378	349
6	251	-230
7	358	314
8	378	-334
9	127	105
10	166	151
11	56	-62
12	<47	25
13	99	102
14	121	129
15	96	121

$\bar{2}$,k,6

0	941	941
1	111	123
2	243	241
3	225	-243
4	<47	17
5	82	58
6	<55	48
7	164	162
8	192	176
9	<63	-54
10	280	246
11	<49	-1
12	103	109
13	82	-86
14	38	52

$\bar{2}$,k,7

1	70	-83
2	130	-138
3	231	244
4	184	-191
5	458	502
6	<53	-15
7	173	170
8	<61	27
9	177	152
10	<58	15
11	<52	12
12	109	-112
13	86	103
14	54	39

$\bar{2}$,k,8

0	156	169
1	251	296
2	232	264
3	191	186
4	275	301
5	112	-133
6	<81	-27
7	182	-181
8	128	139
9	114	-109
10	176	182
11	56	19
12	62	84
13	93	98

$\bar{3}$,k,1

3	356	-347
4	75	93
5	184	-169
6	143	112
7	208	160
8	239	202
9	75	65
10	232	173
11	108	-88
12	81	64
13	67	-56

$\bar{3}$,k,3

0	400	365
1	<59	39
2	165	171
3	<62	50
4	132	-144
5	69	87
6	94	70
7	<73	-63
8	260	243
9	119	-86
10	254	220
11	<60	2
12	216	200
13	<32	49

$\bar{3}$,k,4

1	113	127
2	298	-310
3	158	145
4	90	-88
5	191	192
6	127	112
7	195	207
8	116	98
9	64	-14
10	117	90
11	113	111
12	104	-114
13	85	110

$\bar{3}$,k,5

0	<53	-77
1	338	334
2	170	180
3	174	187
4	243	242
5	<58	41
6	233	213
7	<63	-33
8	79	91
9	69	-73
10	<54	5
11	61	68
12	100	106
13	<19	30

$\bar{3}$,k,6

1	282	294
2	142	141
3	147	155
4	88	-92
5	96	-100
6	61	-43
7	105	86
8	<61	29
9	222	209
10	123	129
11	182	192
12	96	-107

$\bar{3}$,k,7

0	86	81
1	91	102
2	194	195
3	147	-156
4	192	190
5	<56	-37
6	214	204
7	80	71
8	142	127
9	120	122
10	99	-90
11	<32	19
12	<23	-20

$\bar{3}$,k,8

1	182	197
2	166	172
3	<77	7
4	81	120
5	166	150
6	<66	-41
7	156	164
8	200	-213
9	57	78
10	<64	66
11	<40	55

$\bar{4}$,k,3

1	252	253
2	64	96
3	<64	26
4	<63	29
5	62	-19
6	117	-96
7	83	66
8	116	-104
9	149	115
10	<81	9

$\bar{4}$,k,4

0	104	103
1	<59	-11
2	94	99
3	162	-148
4	59	18
5	58	-54
6	78	74
7	129	128
8	135	131
9	79	73

$\bar{4}$,k,5

1	<54	-11
2	<55	39
3	68	69
4	67	55
5	148	130
6	85	-78
7	191	186
8	82	-68
9	<27	18

$\bar{4}$,k,6

0	181	182
1	<54	-6
2	172	159
3	<52	13
4	<51	-61
5	<50	61
6	<45	-13
7	41	-21
8	105	82

$\bar{4}$,k,7

1	<49	-13
2	99	-94
3	91	91
4	48	-40
5	144	128
6	93	62
7	118	106
8	104	81

$\bar{4}$,k,8

0	<72	60
1	151	171
2	116	127
3	121	104
4	95	102
5	<63	-44
6	80	85
7	71	-79

Table 1. For comparison, r.m.s. shifts indicated for the light atoms after the final cycle were

$$[\overline{(\Delta x^2)}]^{\frac{1}{2}}=0\cdot0004,\ [\overline{(\Delta y^2)}]^{\frac{1}{2}}=[\overline{(\Delta z^2)}]^{\frac{1}{2}}=0\cdot0001\ .$$

The thermal parameters are shown in Table 2. The complete set of F_o and F_c is given in Table 3.

Positions of hydrogen atoms

The peaks which were identified as seven hydrogen atoms on our first $0kl(F_o-F_c)$-synthesis persisted on $0kl$ difference-maps calculated at various other stages of the refinement. In the presence of so many heavier atoms with pronounced anisotropic thermal motion it

Table 3. *Positional parameters for hydrogen atoms*

Atom	x_i	y_i	z_i
H_1	0·320	−0·010	0·170
H_2	0·395	0·274	0·117
H_3	−0·217	0·150	−0·094
H_4	0·978	0·400	0·066
H_5	0·911	0·405	0·172
H_6	0·752	0·263	0·259
H_7	0·733	0·337	0·334

Isotropic temperature-factor: $\exp -(2\cdot 4 \sin^2 \theta/\lambda^2)$.

would have been excessively optimistic to attach much significance to the actual peak positions. It was gratifying that the quality of the data was such that indications of the hydrogen atoms were found at all. Their persistence left little doubt that the points of attachment were correctly chosen—namely, H_1 attached to N_1, H_2 to N_2, H_3 to N_3, H_4 and H_5 to O_3, and H_6 and H_7 to O_4. Reasonable coordinates could then be deduced.

From an analysis of the inter-atomic vectors it was unlikely that H_1, H_2 and H_3 were involved in hydrogen bonds at all. H_4 lay on a short $O_3 \cdots O_2$ vector (about 2·7 Å), H_5 on a short $O_3 \cdots O_4$ vector (2·8 Å), H_6 between atoms of types O_4 and O_1 (2·8 Å), and H_7 between O_4 and N_3 (3·1 Å). There were no other short contacts except from N_1 to the water oxygen atom (type O_4) nearest it (3·5 Å) and from N_3 to the O_4 atom of the adjacent unit cell (3·4 Å) in the $\bar{x}$-direction. If H_1 and H_3 were placed, respectively, on these two vectors then it turned out that they were also very close to the planes of the amide groups to which they belonged.

The hydrogen atoms were therefore assumed to lie on the above hydrogen bonds or lines of close approach, and to be at 1·0 Å from the oxygen atoms or 0·97 Å from the nitrogen atoms to which they were attached. This left only H_2, which was placed in the plane of C_1, N_2 and C_2 and roughly on the external bisector of $C_1\hat{N}_2C_2$. The coordinates of the hydrogen atoms were adjusted to maintain their relative positions with respect to the rest of the structure from cycle to cycle. The final coordinates are listed in Table 4.

Atomic scattering factors

For the light atoms, the curves of Berghuis *et al.* (1955) were used in the early calculations, and the Hoerni–Ibers (1954) atomic scattering factors in the final refinement. For potassium and copper, the K^+ and Cu^+ scattering curves of Berghuis *et al.* were corrected for anomalous dispersion by the method of Dauben & Templeton (1955). For copper, the size of this correction was about −2 electrons over the observable range, which was large compared with a slight uncertainty whether the copper should be treated as Cu°, Cu^+ or Cu^{++}. The difference between these would be significant for only a few reflections at low θ's. Chemical intuition suggested that the charge originally on Cu^{++} was partly neutralised by chelation, the resultant charge of −2 being distributed over the peripheral atoms of the complex ion. The use of the Cu^+ curve was further justified by the absence of any peak at the origin of the first $0kl(F_o-F_c)$-projection (Fig. 2), and by the fact that in each cycle the 'atomic' scaling factor (see above) for copper was always close to the average value for the other atoms.

Rate of convergence

The root mean square changes of the parameters (of the atoms other than copper, potassium and hydrogen) during the eight final refinement cycles are shown in Table 4, together with other evidence of the progress of the calculation. For comparison we have also included the equivalent information for six cycles of least-squares refinement in which only the reflections with $h+k+l=(2n+1)$—i.e. those to which the copper atom made no contribution—were used. The purpose of this computational excursion was to see whether the refinement of such a structure could be effectively speeded up by carrying out a partial refinement with reflections minimally affected by a heavy atom whose position was already accurately known. (Note that the thermal parameter changes refer to the coefficients *before* conversion to the standard form.)

It is seen from Table 4 that convergence was achieved after *six* cycles of refinement with the *ca.* 400 *odd* reflections. While R and $\Sigma w(\Delta F)^2$ were in fact smaller than the corresponding quantities for the same reflections treated as part of the complete data, the standard deviations of the atomic parameters were appreciably larger after the shortened refinement than those of the parameters obtained by the eight cycles using all 1260 reflections.

	x_i (Å)	y_i (Å)	z_i (Å)
R.m.s. standard deviations of coordinates from:			
Complete data	0·006	0·007	0·006
Odd F's only	0·008	0·009	0·008
R.m.s. difference between final coordinates	0·009	0·010	0·013
Max. difference between final coordinates	0·018	0·021	0·022

Although the shortened refinement did not produce a value of any parameter which was significantly different from the 'complete data' value, some of the differences were considerably greater than the r.m.s. changes indicated by the final cycle of each sequence. While it is likely that the two sets of parameters would have been reconciled by following the shortened refinement with calculations using the complete data, we did not pursue the matter to this conclusion. It was apparent that this method offered little over-all economy in computations.

Table 4. *Comparison of rates of convergence of refinements carried out, respectively, with the complete data (cycles* 1–8*) and with only the reflections with odd* $(h+k+l)$ *(cycles* 1′–6′*)*

The r.m.s. values of the parameter-changes are calculated for light (C, N, O) atoms only

Refinement cycle	$10^4 \times$ r.m.s. value of Δx_i	Δy_i	Δz_i	$10^3 \times$ r.m.s. value of $\Delta\beta'_i$	$\Delta\alpha'_i$	$\Delta\gamma'_i$	$\Delta\delta'_i$	$\Delta\varepsilon'_i$	$\Delta\eta'_i$	R%	$\Sigma w(\Delta F)^2$
1	51	8	8	47	199	9	17	11	10	12·3	50·54
2	29	5	6	22	84	6	14	17	5	9·5	24·06
3	20	3	4	18	35	5	20	12	4	9·0	19·30
4	14	2	3	10	51	2	17	15	2	8·7	18·34
5	6	1	2	9	20	2	7	4	1	8·6	17·53
6[a]	14	3	4	9	30	1	21	7	3	7·9	17·23
7[b]	10	2	2	10	21	2	10	5	3	8·1(9·4)[d]	15·20(7·37)[d]
8	4	1	1	4	15	2	5	2	1	7·9(9·1)[d]	14·45(6·90)[d]
1′	20	6	6	40	135	9	34	26	10	13·5	18·35
2′	13	3	5	14	25	5	33	18	5	11·3	13·49
3′	9	2	3	15	51	4	13	9	3	10·7	9·54
4′	5	2	1	8	31	3	11	9	3	9·9	8·22
5′[c]	8	2	2	5	38	2	9	5	2	9·3	7·32
6′	8	1	1	13	27	2	8	10	3	9·0	6·19

Superscripts: *a.* Error in H-coordinates detected and corrected. *b.* Unobserved F's given non-zero weights. *c.* H-atoms included in F_c. *d.* Values in parentheses are for *odd* reflections alone

4. Description of structure

We have used sub-script numerals to distinguish atoms of the same type within an asymmetric unit, and super-script numerals for atoms belonging to asymmetric units other than that centred about the copper atom at (0, 0, 0). The relationships between the coordinates of such atoms and those listed in Table 1 are as follows:

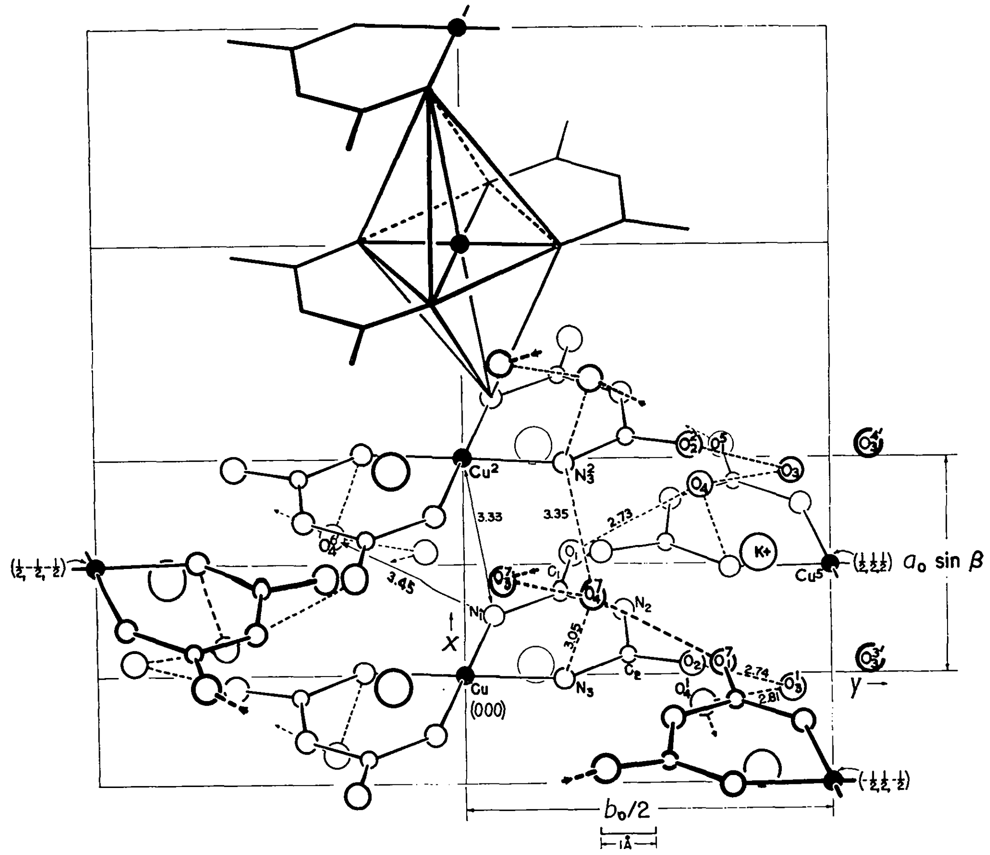

Fig. 3. Structure of $K_2Cu(NHCONHCONH)_2 . 4\,H_2O$ projected along c-axis.

$(^2)$: x_i+1, y_i, z_i; $(^3)$: x_i+1, y_i+1, z_i;
$(^4)$: x_i+2, y_i+1, z_i; $(^5)$: $x_i+\frac{1}{2}, \frac{1}{2}-y_i, z_i+\frac{1}{2}$;
$(^6)$: $x_i+\frac{3}{2}, \frac{1}{2}-y_i, z_i+\frac{1}{2}$; $(^7)$: $x_i-\frac{1}{2}, \frac{1}{2}-y_i, z_i-\frac{1}{2}$;
$(^8)$: $x_i+\frac{1}{2}, \frac{1}{2}-y_i, z_i-\frac{1}{2}$.

Dashes (′) show atoms obtained from the corresponding un-dashed atoms by inversion through the copper atom of the same asymmetric unit. Thus O_3^5, O_4^5, $O_3^{5\prime}$ and $O_4^{5\prime}$ are the oxygen atoms of the four water molecules indicated by the molecular formula, considered with respect to the copper atom Cu^5 at $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$.

The structure is shown in projection along the z-axis in Fig. 3. Inter-atomic vectors and inter-vector angles are listed in Table 5. Safe estimates of the standard deviations are 0·015 Å for the bond-lengths and 2° for inter-bond angles.

Table 5. *Inter-atomic distances and inter-vector angles*

Bond-lengths		Other important distances	
$Cu–N_1$	$1·93_8$ Å	$Cu–Cu^2$	3·84 Å
$Cu–N_3$	$1·93_2$	$N_1–Cu^2$	3·33
$C_1–N_1$	$1·34_2$	$N_1–N_3'$	2·74
$C_1–N_2$	$1·38_2$	$N_1–N_3$	2·73
$C_1–O_1$	$1·24_7$	$O_4–N_3^6$	3·35
$C_2–N_2$	$1·40_9$	$O_4^7–N_3^2$	
$C_2–N_3$	$1·33_7$	$O_4–N_1^{6\prime}$	3·45
$C_2–O_2$	$1·27_4$		

Hydrogen bonds		Environment of K^+ ion	
$O_1 \cdots O_4$	2·73 Å	$K^+–O_2$	2·83 Å
$O_2^2 \cdots O_3$	2·74	$K^+–O_2^2$	2·67
$O_3 \cdots O_4$	2·81	$K^+–O_1^7$	2·67
$O_4 \cdots N_3^5$	3·05	$K^+–O_1^8$	2·89
$O_4^7 \cdots N_3$		$K^+–O_3^{3\prime}$	2·78
		$K^+–O_3^{4\prime}$	2·81

Angles between bonds, hydrogen-bonds and other vectors

$N_1–Cu–N_3$	89·7°	$C_1–N_2–C_2$	132·2°
$N_1–Cu^2–N_3^2$	87·9	$C_1–N_2–H_2$	112·3
$N_1–Cu^2–N_1^2$	89·7	$C_2–N_2–H_2$	115·4
$Cu–N_1–C_1$	131·5	$N_2–C_2–O_2$	115·3
$Cu–N_1–Cu^2$	89·7	$N_2–C_2–N_3$	117·5
$C_1–N_1–Cu^2$	93·1	$O_2–C_2–N_3$	127·3
$Cu–N_1–H_1$	119·5		
$C_1–N_1–H_1$	107·6	$C_1–O_1–O_4$	126·0
		$C_2^2–O_2^2–O_3$	114·1
$Cu–N_3–C_2$	131·3		
$Cu–N_3–O_4^7$	111·9	$O_2^2–O_3–O_4$	104·0
$C_2–N_3–O_4^7$	93·6		
$Cu–N_3–H_3$	112·0	$O_1–O_4–O_3$	110·3
$Cu^2–N_3^2–O_4^7$	111·9	$O_1–O_4–N_3^5$	97·7
$C_2–N_3–H_3$	114·3	$O_3–O_4–N_3^5$	131·3
$C_2^2–N_3^2–O_4^7$	114·9	$O_1–O_4–N_3^6$	126·4
		$N_3^5–O_4–N_3^6$	73·6
$N_1–C_1–N_2$	117·6	$O_3–O_4–N_3^6$	114·5
$N_1–C_1–O_1$	126·1	$O_1–O_4–N_1^{6\prime}$	170·3
$O_1–C_1–N_2$	116·3	$N_3^5–O_4–N_1^{6\prime}$	73·7
		$O_3–O_4–N_1^{6\prime}$	79·2

Environment of copper atom

The copper atom is bonded to four nitrogen atoms lying at distances of 1·93 Å and at the corners of an almost perfect square of side 2·73 Å. Adjacent complexes are stacked along lines parallel to the x-axis, with their 'coordination-squares' at such an inclination that each bonded nitrogen atom N_1 also lies in the octahedral position with respect to the copper atom of one of the two adjacent complexes. The environment of the metal atom is thus comparable with that in copper phthalocyanine (Robertson, 1935, 1936, 1937). The octahedra of nitrogen atoms about Cu and Cu^2 share the $N_1–N_1^{2\prime}$ edge. The distance between the copper atom and the octahedral nitrogen is the minimum distance between any two points in adjacent complexes, but its size (3·33 Å) implies that any inter-action between these atoms is weak.

Dimensions of ligand molecule

The biuret molecule has previously been studied in the crystals of biuret hydrate (Hughes, Yakel & Freeman, 1961) and *bis*-biuret cadmium(II) chloride (Cavalca, Nardelli & Fava, private communication). The dimensions found in the hydrate and in the copper complex are shown in Fig. 4; the following discussion applies substantially also to the cadmium derivative.

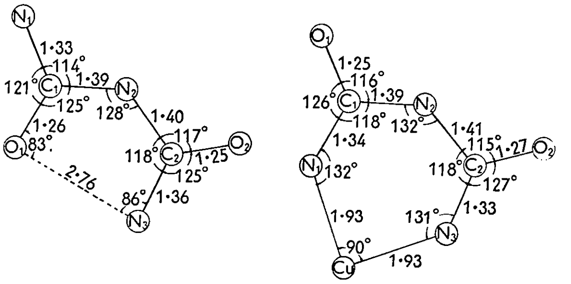

Fig. 4. Comparison of dimensions of biuret in (*left*) the hydrate and (*right*) the copper complex.

Coordination to copper seems to have no significant effect upon the linear and angular dimensions of the biuret molecule, although the configuration of the two amide groups is changed. There is thus no detectable change in the relative contributions of the resonance forms of the biuret molecule (Hughes *et al.*, 1961). Neither in biuret nor in the copper complex are there significant differences between the lengths of equivalent C–N bonds ($C_1–N_1$ and $C_2–N_3$; $C_1–N_2$ and $C_2–N_2$). In the complex, the difference between the bond-lengths of $C_1–O_1$ and $C_2–O_2$ lies just inside the limit of 'possible significance' according to the usual t_0-test (Cruickshank & Robertson, 1953).

The value of the angle $C_1–N_2–C_2$ is abnormally large in the complex (132°) as it is also in the free molecule (128°). The cause in both cases is almost certainly steric strain—arising in the free molecule from an internal hydrogen bond (Hughes *et al.*, 1961), and in the complex from the accommodation of the metal

Table 6. *Details of planes of best fit*

Each plane is represented by $lx'+my'+nz'+k=0$ with respect to orthogonal axes, relative to which the usual monoclinic coordinates of a point (x, y, z) become $x'=x+z\cos\beta$, $y'=y$, and $z'=z\sin\beta$

					Angles between normal to plane and recip. axes			
Plane	l	m	n	k	x^*	y^*	z^*	Angle between plane and plane 1
1	+0·8666	−0·1221	−0·4838	0	33·9°	97·0°	118·9°	—
2	+0·8885	−0·1123	−0·4450	+0·0896	—	—	—	2·7°
3	+0·8597	−0·0595	−0·5073	+0·1287	—	—	—	3·9
4	+0·8770	−0·0817	−0·4735	+0·0643	32·75	94·7	118·3	2·5

	Deviations (in Å) from plane				
Atom	1	2	3	4	Max. standard deviations of atomic positions (in Å)
Cu	0	—	—	—	—
C_1	—	+0·0046	—	+0·0093	0·0075
O_1	—	−0·0017	—	−0·0272	0·0055
N_1	0	−0·0015	—	−0·0165	0·0066
N_2	—	−0·0014	+0·0005	+0·0627	0·0066
C_2	—	—	−0·0018	+0·0042	0·0075
O_2	—	—	+0·0007	−0·0364	0·0055
N_3	0	—	+0·0006	+0·0039	0·0066

Key to planes. Plane 1: $CuN_1N_3(N_1'N_3')$. Plane 2: $C_1O_1N_1N_2$. Plane 3: $C_2O_2N_2N_3$. Plane 4: $C_1O_1N_1N_2C_2O_2N_3$

atom. Similarly the internal Cu–N–C angles at both N_1 and N_3 are 131°, a considerable distortion from the value for trigonal bonds.

Configurations at ligand nitrogen atoms

Apart from its bonds with Cu and C_2, the atom N_3 has two short contact distances, each with a water oxygen atom of type O_4 (see Fig. 3). These atoms complete a distorted tetrahedron about N_3. We have assumed that the hydrogen-bond $N_3 \cdots H–O_4^7$ (3·05 Å) involves the hydrogen atom H_7^7 of the water molecule. The amide hydrogen H_3 is then not used in any hydrogen bond: we have placed it on the second $N_3 \cdots O_4$ vector (3·35 Å), which lies almost in the CuN_3C_2 plane. Apart from its length, this vector is precluded from being a hydrogen bond by the angles which it makes with the three certain hydrogen-bonds at O_4. It is equally possible that H_3 should have been placed along the bond $N_3–H \cdots O_4^7$, and that H_7^7 lies roughly in the direction $O_4^7 \cdots N_3^2$ (but at reasonable angles with the other bonds to O_4^7). The positions of N_3 and O_4 would be scarcely affected, and this arrangement would leave N_3 with a more satisfying, distorted trigonal arrangement of three bonded contacts (bond-angles 131°, 112° and 94°).

The configuration about N_1 is similarly a distorted triangle, consisting in this case of Cu, C_1 and H_1. The $N_1–H_1$ bond does not take part in hydrogen bonding, and the most reasonable position for H_1 is close to the plane of the amide group. The copper atom Cu^2 of the adjacent complex lies almost exactly on the normal to both the Cu–N_1 and C_1–N_1 bonds.

Planarity of amide groups

Just as the free biuret molecule is not planar, so the coordinately-bound biuret residue is bent slightly about the line joining Cu to N_2, the two amide groups then being rotated about their C–N_2 bonds until their normals are inclined at 4° 56′ to each other. Neither of the two planar amide groups thus lies in the plane of the 'coordination-square'. If a plane of best fit is calculated for the *whole* biuret residue, the atom N_2 is found to lie 0·06 Å (about ten times the coordinate standard deviation) away from it. Even with this approximation, however, the complex is bent by 2° 30′ along the N_1N_3 edges of the coordination-square.

In Table 6, we list the coefficients for the planes fitted to various sets of atoms by the least-squares method of Schomaker, Waser, Marsh & Bergman (1959). The table also includes the distances of the atoms from the planes fitted to them, and the angles between the reciprocal axes and the normals to two of the least-squares planes.

Atoms not involved in complex

The two independent water molecules and the potassium ion fit into the structure in interesting ways without, however, suggesting that any general conclusions are to be drawn from them.

The potassium ion is in a hole in the structure, its nearest neighbours being the six oxygen atoms shown in the list of K^+–O distances in Table 5. The six oxygen atoms form a slightly distorted trigonal prism with the potassium ion almost at the centre. Successive prisms in the x-direction share their triangular faces. The O–K^+–O angles subtended at the potassium ion by the long edges of the prism all lie between 87·0° and 88·7°, and those angles subtended by the edges of the triangular faces between 75·0° and 79·1°.

One corner of each of these triangular faces is occupied by a water molecule whose oxygen atom is

Table 7. *Parameters of thermal vibration ellipsoids*

q_i, magnitudes of principal axes; g_{ik}, direction cosines with respect to reciprocal axes k; φ_i, angles between principal axes and normal to plane of best fit through biuret molecule (for Cu, φ_i is referred to normal to coordination square)

Atom	Axis i	q_i	g_{i1}	g_{i2}	g_{i3}	φ_i
C_1	1	0·674	0·861	0·135	0·551	63·2°
	2	0·506	0·066	0·927	−0·364	79·8
	3	0·247	−0·504	0·350	0·751	151·1
C_2	1	0·636	0·630	0·090	0·814	84·1
	2	0·510	−0·521	0·785	0·296	132·9
	3	0·222	0·576	0·613	−0·500	43·6
N_1	1	1·011	0·768	−0·173	−0·560	8·1
	2	0·492	0·581	0·593	0·598	81·8
	3	0·334	−0·269	0·787	−0·573	89·6
N_2	1	0·812	0·762	0·039	−0·591	10·4
	2	0·519	0·629	0·196	0·796	83·7
	3	0·372	−0·156	0·980	−0·136	98·3
N_3	1	0·694	0·977	−0·021	−0·141	20·5
	2	0·512	−0·129	0·732	−0·677	79·1
	3	0·345	0·169	0·681	0·723	107·1
O_1	1	1·131	0·815	0·217	−0·478	17·2
	2	0·658	−0·388	0·893	−0·255	106·1
	3	0·098	0·431	0·395	0·824	96·0
O_2	1	1·097	0·941	0·070	−0·263	15·7
	2	0·442	0·093	0·887	0·457	103·7
	3	0·291	0·325	−0·456	0·850	97·6
O_3	1	1·240	0·987	−0·122	0·179	38·6
	2	0·752	−0·050	0·409	0·906	124·3
	3	0·583	0·156	0·905	−0·385	74·8
O_4	1	1·202	0·865	0·299	0·465	60·9
	2	0·931	−0·030	0·833	−0·553	78·3
	3	0·610	−0·502	0·466	0·692	148·2
K^+	1	0·701	0·816	0·325	−0·419	23·7
	2	0·632	0·148	−0·917	−0·359	66·6
	3	0·425	0·559	−0·232	0·834	86·4
Cu	1	0·767	0·896	0·077	−0·372	13·5
	2	0·347	−0·058	0·997	0·527	101·6
	3	0·289	0·440	−0·026	0·927	96·9

of the type O_3. As a consequence of the alternation of potassium ions and triangles of oxygen atoms, each O_3 has two K^+-ions as near neighbours at distances of 2·76 and 2·81 Å. An approximate tetrahedron about O_3 is completed by the two atoms, O_4 and O_2^2 to which it is hydrogen-bonded. [It should be noted by reference to Table 5 that O_3 is not one of the near neighbours of the potassium ion labelled K^+ in Fig. 3, but is so related to the ions labelled $K^{3'}$ and $K^{4'}$.]

The oxygen atom O_4 of the second water-molecule also has an approximately tetrahedral environment. It is hydrogen-bonded to O_3, O_1 and N_3^5, its hydrogen atoms lying on or near the lines joining it to the latter two.

Analysis of thermal parameters

The parameters q_i and g_{ik} of the thermal vibration ellipsoids for all atoms (other than hydrogens) are shown in Table 7. They were derived from the thermal coefficients listed in Table 2 by the procedure of Rollett & Davies (1955) and represent, respectively, the magnitudes of the principal axes of the vibration ellipsoids and their direction cosines with respect to the reciprocal axes.

We have also calculated the angles between the same principal axes and the normal to the plane of best fit of the biuret residue (plane 4 in Table 6); for the copper atom the direction cosines are referred to the normal to the coordination-square.

The results are in agreement with expectation. The atoms of the complex ion have their maximum amplitudes of vibration roughly normal to the plane of the complex. For the water molecules and potassium ion no such relationship is expected or found. The heavy atoms, copper and potassium, have relatively small amplitudes; the two loosely bound water molecules have the largest amplitudes of vibration. In the biuret residue, the two carbon atoms are exceptional in that, of all the major vibration-axes, theirs are the shortest and do not lie near the normal to the plane of the molecule.

It seems as though, superimposed on the general vibration of the complex as a whole, there is a 'folding' mode in which the copper atom and the two carbon atoms of each biuret residue are stationary and the complex bends along the N_1N_3-edges of the coordination-square. In this mode the atoms O_1', N_2', O_2', N_1 and N_3 move 'up' while the atoms N_1', N_3', O_1, N_2 and O_2 move 'down', and vice versa.

5. Colour

To a first approximation, the copper atom in this crystal experiences a tetragonal ligand field. The situation is analogous to that in copper phthalocyanine, for which an energy-level scheme compatible with electron resonance measurements has been proposed by Gibson, Ingram & Schonland (1958). Light absorption in the metal phthalocyanines is, however, a function of the conjugated bond system rather than of the metal atom, so that the analogy cannot be taken further.

The absorption spectrum of crystalline potassium *bis*-biureto cuprate(II) tetrahydrate has a maximum at 505 mμ and is otherwise almost identical with the spectrum of the compound in aqueous solution between 375 mμ and 640 mμ (Dr J. Ferguson, private communication). The complex therefore preserves its structure in solution, so that it is possible to confirm that light absorption at $\lambda_{\text{max.}} = 505$ mμ is associated with copper(II) coordinated by four peptide nitrogen atoms (Kober & Haw, 1916; Plekhan, 1952; Kato, 1959).

The amide nitrogen atoms, after the ionization of the protons, are not only negatively charged but are presumably also more polarizable than the nitrogen atoms in ammonia and ethylene diamine. It is therefore not necessary to invoke stabilization by π-bonding (Kato, 1959) to explain why the copper(II) complex of biuret absorbs at a shorter wave-length than do $Cu(NH_3)_4^{++}$ ($\lambda_{\text{max.}} = 600$ mμ) and $Cu\,en_2^{++}$ ($\lambda_{\text{max.}} = 550$ mμ).

6. Discussion

The conclusions to which this structure analysis leads us are:

(1) In the complex formed by biuret and copper in alkaline solution, the copper atom is bonded to the nitrogen atoms of the amide groups. This confirms the structure first proposed for this complex by Tschugaeff (1907), and supports the view (Dobbie & Kermack, 1955; Datta & Rabin, 1956; Rabin, 1956, 1958) that in alkaline solution metal–peptide binding at peptide nitrogen atoms follows the ionization of the peptide hydrogen atoms.

(2) The ligand molecule in this complex retains its essential dimensions after coordination. In particular, the dimensions and planarity of the amide-groups are unchanged. The amide-group resonance energy is consequently not reduced by coordination.

(3) On the other hand, distortion to accommodate the metal atom occurs by the twisting of the amide groups relative to each other, and by the variability of the internal bond angles at the ligand nitrogen atoms.

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