The Crystal Structure of Biuret Hydrate*

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The hydrate of biuret, $NH_2CONHCONH_2$, has a unit cell with space-group $C_{2h}^5-P2_1/c$ and

$$a = 3{\cdot}63, \quad b = 17{\cdot}78, \quad c = 9{\cdot}18 \text{ Å}; \quad \beta = 119° 30'$$

and $n = 4$. The planar amide groups in biuret have normal dimensions and are slightly inclined and *trans* to each other, with an internal hydrogen-bond. The biuret molecules lie in buckled sheets parallel to (100), one molecule being joined to each of three neighbours by $N–H \cdots O$ hydrogen-bonds.

Tunnels run through the structure parallel to the *y*-axis. Water molecules form zig-zag chains along these tunnels, alternate sites lying across apparent centres of symmetry. Under the conditions of the present experiments, only three-fifths of the available water-sites are filled. The water-molecules are loosely hydrogen-bonded to amide oxygen atoms of the biuret structure, but the distances between adjacent water sites are remarkably short (2·48 Å).

Introduction

Biuret, $NH_2CONHCONH_2$, can be crystallized from alcohol in white, rectangular plates whose preparation, properties and reactions have recently been reviewed by Kurzer (1956). Haworth & Mann (1943) have published some crystal data obtained by Hargreaves & Taylor. With 8 molecules per unit cell, the low symmetry of the space-group (C_{2h}^6-A2/a or C_s^4-Aa) deterred these authors from a crystal structure analysis.

Anhydrous biuret is hygroscopic (Wiedemann, 1848) and, when the substance is crystallized from water, a hydrate is formed. This was formerly believed to be a monohydrate, though it was known that the water of crystallization might be partly lost on storage at room temperature (Behrend & Schultz, 1909) and completely at 110 °C. (Hofmann, 1871). More recently, Rollet & Cohen-Adad (1951) have found that at room temperature the composition of the freshly formed hydrate is 5 biuret:4 H₂O (or biuret.0·8 H₂O).

The structure of biuret hydrate has now been examined partly as a contribution to the small list of structure-determinations of compounds with terminal amide groups, partly out of an interest in the hydrogen-bond systems of amides, and partly because this substance is the simplest compound which undergoes the characteristic polypeptide 'biuret-reaction'.

Experimental

Biuret hydrate crystals were obtained as white, lath-like needles by slow evaporation from aqueous solution. They showed marked cleavage only in the (100) plane, and effloresced before melting (indicating that they were hydrated). No chemical analysis was carried out. Two crystals were finally selected for systematic examination. Their physical dimensions ($a \times b \times c$, $a < c$) were $1{\cdot}43 \times 0{\cdot}05 \times 0{\cdot}3$ mm., 118°, and $0{\cdot}243 \times 0{\cdot}033 \times 0{\cdot}153$ mm., 118°, respectively. The first crystal was used for rotation, oscillation and Weissenberg zero-, first- and second-layer photographs about the *a*-axis, and was then reset for recording the ($h0l$)-zone. The second crystal was used to obtain *c*-axis data for $l = 0$ to 5. All photographs were taken on Kodak (No Screen (Medical)) film, using nickel-filtered Cu $K\alpha$ radiation.

The intensities were measured on multiple-film Weissenberg photographs by visual comparison with calibrated intensity-strips (Robertson, 1943) and were corrected for the Lorentz and polarization factors. The ($0kl$)-intensities were originally scaled by Wilson's method (1942), and the other intensities were converted to the same scale. The scale-factor was continually revised during the course of the refinement.

Space group and unit cell

The systematic absences were: $0k0$ for k odd and $h0l$ for l odd. Hence the space group was unequivocally $C_{2h}^5-P2_1/c$. The unit cell dimensions a, c and β were fitted by least-squares to the spacings recorded for 12 intense $h0l$-reflections; b was calculated from a

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single high-angle reflection, (0,18,0). The spacings were measured on oscillation photographs taken in a 5 cm. radius rotation camera, with NaCl powder patterns as internal calibration. The values were:

$$a = 3 \cdot 63_0 (\pm 0 \cdot 003), b = 17 \cdot 78 (\pm 0 \cdot 01), c = 9 \cdot 18 (\pm 0 \cdot 01) \text{ Å},$$
$$\beta = 119° 30' (\pm 10'),$$

whence

$$V = 515 \cdot 7 (\pm 1) \times 10^{-24} \text{ cm.}^3$$
$$[\lambda (\text{Cu } K\alpha) = 1 \cdot 5418 \text{ Å}, \ d_{100} (\text{NaCl}) = 5 \cdot 6285 \text{ Å.}]$$

The density of the crystals was measured by flotation in benzene-iodobenzene and bromobenzene-carbon tetrachloride mixtures at room temperature. The mean of 4 determinations was: $\varrho = 1 \cdot 5200 (\pm 0 \cdot 004)$ g.cm.$^{-3}$.

From the formula weight (121·1) of biuret monohydrate, $NH_2CONHCONH_2 \cdot H_2O$, the calculated density was 1·559 g.cm.$^{-3}$ for $n = 4$. In reverse, the measured unit cell volume and density led to a formula weight of 118 for the asymmetric unit. The real significance of these discrepancies was not appreciated at first.

Trial structure

The shortness (3·63 Å) of the unit-cell edge in the x-direction, coupled with the high intensity of the (100)-reflection, made it probable that the biuret molecules were essentially flat and lay parallel to the (100)-plane. Trial structures were obtained by moving a reasonable scale drawing of a projection of the expected molecule around a yz-section of the unit cell, on the assumption that—by analogy with crystals of other amides (Donohue, 1952)—the number of hydrogen atoms not involved in hydrogen bonds would be minimal.

The correct model turned out to be one with an initial reliability factor, $\Sigma ||F_o| - |F_c||/\Sigma |F_o|$, of 44%. A synthetic Patterson projection agreed well with the real projection.

The convention adopted for the labelling of the atoms for all further calculations was as follows:

$$N_1 - C_1 \overset{\displaystyle N_2 - C_2 \overset{\displaystyle O_2}{\underset{\displaystyle N_3}{\diagup\diagdown}}}{\underset{\displaystyle O_1}{\diagup\diagdown}}$$
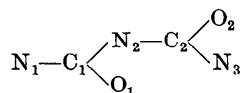
The oxygen atom of the water molecule was designated O_3.

Refinement of (0kl)-projection

The first Fourier projection calculated from those 87F_{0kl}'s for which the trial structure yielded reasonably certain signs, contained only one objectionable feature: the projected bond-length of C_2–N_2 was 1·72 Å, which was impossibly large. After four cycles of Fourier refinement, carried out on X-RAC, all bonds had reasonable projected lengths. The (0kl)-projection obtained from a numerical recalculation of

the final X-RAC Fourier map is shown in Fig. 1. With a thermal parameter $B = 1 \cdot 67$ Å^{-2} and using the McWeeny (1951) curves for C, N, and O, the reliability factor was 29% for the observed reflections alone. The agreement improved during two cycles of least-squares refinement ($R = 24$ and 22%, respectively, for the observed reflections, and $B = 1 \cdot 97$ Å^{-2}), but a further cycle yielded no significant improvement.
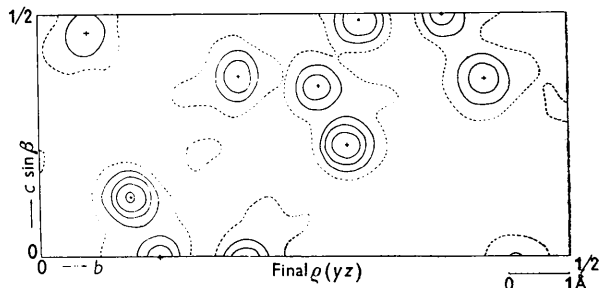

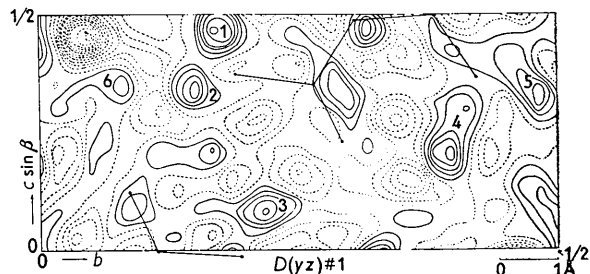Fig. 1. Final x-axis electron-density projection. Broken contour: 1·8 e.; contour interval 1·5 e.

Fig. 2. x-axis $(F_o - F_c)$-projection of partially refined structure Contours at 0·25 e., zero and negative contours broken. Numbered peaks indicate hydrogen atoms.

An $(0kl) - (F_o - F_c)$-synthesis computed at this stage is shown in Fig. 2. Apart from small coordinate shifts it gave clear indications of the positions of six out of the seven hydrogen atoms in the asymmetric unit. Its most interesting feature, however, was the large negative peak at $y = 0 \cdot 045$, $z = 0 \cdot 46$, which showed that the weight of the oxygen atom of the water molecule had been overestimated by about 3 electrons.

This unexpected indication was, in fact, a crystallographic confirmation of the (then) recently-published vapour-pressure/composition study by Rollet & Cohen-Adad (1951). Their figure for the composition of the stable phase of biuret hydrate freshly crystallized from water solution at room temperature was biuret $- (0 \cdot 8) H_2O$. The corresponding formula-weight, 117·5, was in much better agreement with the experimental value of $118 \cdot 0 (\pm 0 \cdot 3)$ than was the formula-weight of the monohydrate, 121·1.

Now if some water molecules were missing, in a regular pattern, there should be some evidence for larger repeat distances than those observed. An effect of this kind has been observed recently by Cant (1956)

in the structure of *cyclo*-hexaglycyl hydrate. But no spots additional to those already indexed were visible on the diffraction photographs of biuret hydrate and it was therefore concluded that the water molecules present were distributed in some statistical fashion over the available sites (see 'Description of structure').

Although a deficiency of about 0·4 H_2O would have been deduced from the difference-synthesis, the atomic scattering curve for O_3 was provisionally multiplied by the factor of 0·8 suggested by the density and the results of Rollet & Cohen-Adad (1951). A recalculation of the $0kl$-structure factors, including the hydrogen atoms, gave an immediate and significant reduction of R(18·2% for the observed reflections, 21·5% over-all).

Determination of x-coordinates

The b- and c-axis Patterson projections were completely dominated by the numerous vectors nearly parallel to (100) between the approximately co-planar atoms of the biuret molecules. There was no evidence that these molecules were inclined at any large angle to the (100)-plane, and there were no vectors identifiable as vectors to the water molecule. In the $(hk0)$-Patterson projection, peaks at (0·14, 0·39) and (0·14, 0·5) suggested that symmetry-related pairs of biuret molecules lay at $x = \pm 0·07$ or $\pm 0·43$.

The approximate x-coordinate of the water–oxygen could be predicted from the position of the O_3-peak on the $(0kl)$-projection. The closeness of this peak to the symmetry centre at $y=0$, $z=\frac{1}{2}$—when considered together with the short a-axis of 3·63 Å—implied that the water molecules formed a zig-zag chain hydrogen-bonded across the apparent centres at $(0, 0, \frac{1}{2})$ and $(\frac{1}{2}, 0, \frac{1}{2})$. If alternate hydrogen-bonds were assumed to be equal in length, then O_3 had to be placed at $\pm (0·2 \ or \ 0·7, \ 0·045, \ 0·46)$.

There were, in fact, four non-equivalent sets of combinations of the respective x-coordinates of the biuret and water molecules. After much trial and error it became apparent that the choice $x_{\text{biuret}} = 0·57$, $x_{H_2O} = 0·20$ was the correct one.

Refinement of structure

The disorder associated with the water-molecules gave rise to pessimism regarding the possibility and meaningfulness of a highly accurate refinement of the structure. It was accordingly decided to make use of only the $(0kl)$-, $(1kl)$-, $(h0l)$- and $(hk0)$-data.

Generalized x-axis first-layer projections—$C_1(y, z)$ and $S_1(y, z)$—were helpful in confirming the general correctness of the structure and the reality of the water-deficiency. The heights of the O_3-peaks on both projections, when divided by appropriate trigonometrical functions, showed that 0·8 H_2O was still an over-estimate of the water content.

The remaining refinement was carried out by means

of the least-squares method using the high speed digital computer, SILLIAC. Non-diagonal terms of the least-squares matrix were neglected with the exception of those depending on the inter-actions of the x- and z-coordinates of each atom. The weighting system was that of Hughes (1941). The temperature factor had the form

$$\exp \left(-B \sin^2 \theta / \lambda^2 - \alpha h^2 \right)$$

so as to take some account of the expected anisotropy of the thermal vibration. No significance was expected to be attached to the magnitude of α.

Fig. 3. Final x-axis $(F_o - F_c)$-projection. Contours at intervals of 0·25 e.; the zero- and negative-contours are broken. Atomic positions for C, N and O are shown by ●, for H by ×.

After five refinement cycles, R for all reflections had dropped from 36·3 to 19·3%. An $(0kl) - (F_o - F_c)$ projection now showed that the weight attributed to O_3 was still too great by 0·2 of an oxygen atom. The reduction of the oxygen atomic scattering curve by this amount caused an immediate reduction of 2% in R. A further improvement of 0·5% resulted from the
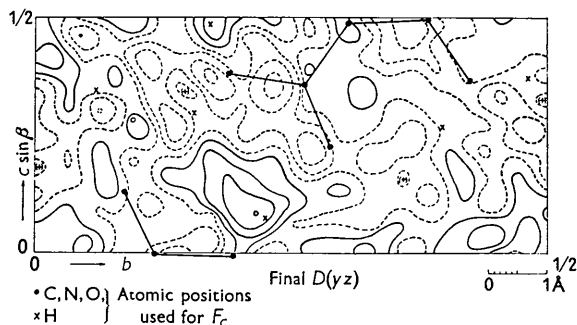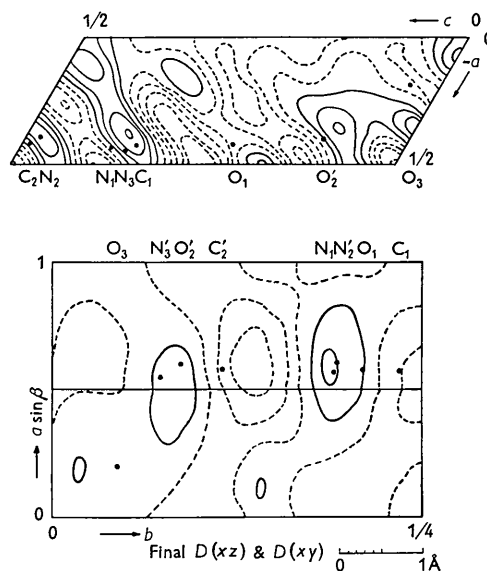

Fig. 4. Final y- and z-axis $(F_o - F_c)$-projections. Contours at intervals of 0·5 e.; the zero- and negative-contours are broken. Atomic positions are shown by ●.

introduction of the hydrogen atoms at their expected positions. No evidence was found in any of the difference-syntheses for the position of the second water–hydrogen atom, and it has not been included in any of our calculations. The shifts of the other atoms as a result of the change in the O_3-contribution and the insertion of the hydrogens were negligible. The final difference-syntheses are shown in Figs. 3 and 4.

If three strong reflections at small θ's ($F_{011}, F_{100}, F_{110}$) were excluded from the final list on the usually

acceptable grounds that the observed intensities were reduced by extinction, then the final $R = 15\cdot7\%$. The reliability-factors for the individual zones were: $R(0kl)$, $12\cdot9\%$; $R(h0l)$, $25\cdot2\%$; $R(hk0)$, $16\cdot0\%$; $R(1kl)$, $15\cdot1\%$. The agreement for the $F(h0l)$'s could probably be further improved by making an absorption correction.

Observed and final calculated structure factors are shown in Table 1.

Table 1. List of structure factors for biuret hydrate

Each group is headed by the values of h and k; each line shows l, $10F_o$ and $10F_c$

0 0
+2	287	+ 198
+4	105	- 79
+6	130	+ 119
+8	232	+ 236
+10	<22	- 6

1 0
+0	894	-1110
+2	<20	+ 8
+4	107	+ 96
+6	<30	- 10
+8	133	- 145

2 0
+0	187	+ 205
+2	<28	+ 6
+4	<29	- 14
+6	<18	+ 0

3 0
+0	<35	- 12
+2	<27	- 23
+4	<13	- 3

4 0
| +0 | 37 | - 17 |

0 1
+1	236	+ 341
+2	284	+ 362
+3	167	- 140
+4	82	+ 89
+5	46	- 48
+6	95	- 100
+7	139	+ 146
+8	89	+ 98
+9	51	+ 52
+10	<22	+ 20

1 1
+0	366	+ 478
+1	305	- 301
+2	143	- 148
+3	<21	+ 26
+4	34	- 31
+5	60	- 59
+6	83	+ 98
+7	98	- 112
+8	<20	+ 12

2 1
| +0 | 294 | - 327 |

3 1
| +0 | 134 | + 114 |

4 1
| +0 | <14 | - 24 |

0 2
+0	353	- 366
+1	<13	- 7
+2	191	+ 183
+3	236	- 242
+4	139	- 144
+5	83	+ 79
+6	<23	+ 11
+7	36	+ 34
+8	56	- 57
+9	<22	+ 11
+10	<22	- 11

1 2
+0	350	+ 351
+1	234	+ 188
+2	40	+ 88
+3	131	+ 138
+4	123	+ 142
+5	<19	- 30
+6	<20	- 15
+7	25	+ 22
+8	53	+ 57

2 2
| +0 | 70 | - 92 |

3 2
| +0 | <30 | - 13 |

4 2
| +0 | <14 | + 8 |

0 3
+1	244	- 217
+2	140	- 160
+3	307	- 394
+4	<18	- 14
+5	125	- 143
+6	142	+ 167
+7	72	- 77
+8	34	- 22
+9	<22	+ 21
+10	<22	- 1

1 3
+0	83	- 56
+1	23	+ 8
+2	113	+ 111
+3	62	+ 69
+4	39	- 47
+5	74	+ 75
+6	43	- 47
+7	43	+ 40
+8	36	+ 42

2 3
| +0 | <26 | - 36 |

3 3
| +0 | 30 | + 10 |

4 3
| +0 | <13 | + 3 |

0 4
+0	56	+ 9
+1	127	- 130
+2	135	- 192
+3	134	+ 119
+4	26	+ 34
+5	166	- 183
+6	69	- 48
+7	43	- 52
+8	34	- 35
+9	50	- 31
+10	<22	- 39

1 4
+0	48	+ 45
+1	217	+ 162
+2	94	+ 88
+3	193	- 213
+4	47	+ 17
+5	<20	+ 22
+6	77	+ 77
+7	<18	+ 3
+8	<13	+ 9

2 4
| +0 | <26 | - 13 |

3 4
| +0 | <30 | - 10 |

4 4
| +0 | <11 | + 2 |

0 5
+1	315	- 353
+2	146	+ 163
+3	291	+ 309
+4	<18	+ 22
+5	110	+ 98
+6	34	- 20
+7	<25	- 25
+8	54	+ 72
+9	91	- 82

1 5
+0	16	- 26
+1	78	+ 95
+2	173	- 161
+3	143	- 145
+4	54	- 49
+5	55	- 69
+6	33	+ 39
+7	<17	+ 16
+8	<13	- 7

2 5
| +0 | 79 | - 82 |

3 5
| +0 | <29 | + 32 |

4 5
| +0 | < 6 | - 4 |

0 6
+0	384	- 375
+1	200	+ 219
+2	130	+ 118
+3	208	- 204
+4	220	+ 230
+5	<24	+ 6
+6	34	- 39
+7	<25	- 38
+8	78	- 64
+9	41	+ 22

1 6
+0	227	+ 207
+1	85	- 89
+2	110	- 109
+3	123	+ 136
+4	131	- 132
+5	28	- 22
+6	27	+ 31
+7	<17	+ 9
+8	40	+ 42

2 6
| +0 | 47 | - 59 |

3 6
| +0 | <29 | + 5 |

0 7
+1	218	- 180
+2	204	+ 207
+3	114	+ 108
+4	128	+ 117
+5	103	+ 95
+6	<23	- 16
+7	109	- 133
+8	<23	+ 9
+9	<18	+ 11

1 7
+0	103	- 100
+1	193	+ 193
+2	101	- 100
+3	119	- 96
+4	<19	- 10
+5	<20	- 10
+6	<19	+ 20
+7	68	+ 85
+8	<19	+ 12

2 7
| +0 | <28 | - 1 |

3 7
| +0 | <29 | - 4 |

0 8
+0	503	- 426
+1	205	- 188
+2	48	- 48
+3	121	+ 123
+4	54	+ 59
+5	105	- 115
+6	<25	+ 22
+7	34	- 34
+8	77	- 58
+9	23	- 35

1 8
+0	236	+ 190
+1	30	+ 51
+2	23	- 15
+3	47	- 42
+4	27	- 49
+5	39	+ 55
+6	26	- 41
+7	46	- 48
+8	<17	+ 31

2 8
| +0 | <29 | - 34 |

3 8
| +0 | <29 | + 4 |

0 9
+1	221	+ 215
+2	38	+ 31
+3	88	+ 91
+4	51	- 39
+5	<23	+ 9
+6	<25	+ 33
+7	77	+ 80
+8	<23	- 8
+9	22	- 5

1 9
+0	284	- 244
+1	118	- 94
+2	38	- 39
+3	26	+ 23
+4	43	+ 48
+5	<19	+ 9
+6	<18	- 13
+7	35	- 28
+8	<17	- 21

2 9
| +0 | 82 | + 139 |

3 9
| +0 | 39 | - 51 |

0 10
+0	358	+ 311
+1	174	+ 173
+2	<22	- 5
+3	<21	- 15
+4	58	+ 47
+5	43	- 26
+6	<25	- 1
+7	54	- 55
+8	100	+ 97
+9	<14	+ 13

1 10
+0	324	- 270
+1	94	- 97
+2	<18	- 25
+3	27	+ 36
+4	102	- 99
+5	<19	+ 7
+6	24	- 31
+7	<19	+ 25

2 10
| +0 | 52 | + 75 |

3 10
| +0 | <26 | + 17 |

0 11
+1	132	+ 148
+2	61	- 61
+3	65	+ 61
+4	84	+ 92
+5	57	+ 53
+6	<25	+ 5
+7	<22	+ 4
+8	<18	+ 14
+9	< 8	+ 19

1 11
+0	67	+ 61
+1	83	+ 68
+2	78	+ 63
+3	47	+ 45
+4	39	+ 25
+5	42	- 30
+6	<20	+ 3
+7	<18	- 11

2 11
| +0 | 52 | - 57 |

3 11
| +0 | <24 | + 22 |

0 12
+0	41	- 58
+1	157	- 158
+2	51	- 49
+3	92	- 84
+4	250	- 262
+5	107	+ 118
+6	<23	+ 16
+7	78	+ 58
+8	<15	+ 15

1 12
+0	25	- 19
+1	130	+ 130
+2	<20	- 17
+3	124	+ 118
+4	122	+ 131
+5	<18	- 26
+6	26	- 46
+7	<17	- 32

2 12
| +0 | <30 | + 20 |

3 12
| +0 | <23 | + 6 |

0 13
+1	84	+ 89
+2	23	- 33
+3	270	- 238
+4	43	- 49
+5	161	- 158
+6	71	+ 44
+7	<18	+ 27
+8	<14	- 29

1 13
+0	32	+ 30
+1	38	- 34
+2	39	+ 42
+3	155	+ 178
+4	<19	- 17
+5	132	+ 142
+6	<13	- 19
+7	<13	+ 3

2 13
| +0 | <30 | - 2 |

3 13
| +0 | <21 | - 1 |

0 14
+0	<30	- 2
+1	<23	+ 12
+2	152	- 162
+3	50	+ 41
+4	57	- 31
+5	55	- 38
+6	51	- 54
+7	<18	+ 3
+8	<11	+ 2

1 14
+0	27	- 33
+1	28	- 17
+2	83	+ 80
+3	114	- 110
+4	<18	- 12
+5	<16	- 13
+6	27	+ 26

2 14
| +0 | <29 | + 27 |

3 14
| +0 | <18 | - 1 |

0 15
+1	<25	+ 10
+2	113	- 115
+3	107	+ 101
+4	22	- 17
+5	53	+ 37
+6	<18	- 7
+7	<15	+ 5

1 15
+0	<20	+ 33
+1	28	+ 6
+2	<20	+ 21
+3	42	- 30
+4	24	- 22
+5	33	- 20
+6	<17	- 29

2 15
| +0 | <28 | + 7 |

3 15
| +0 | <14 | - 3 |

0 16
+0	<32	+ 35
+1	<25	+ 22
+2	107	+ 94
+3	50	- 31
+4	<21	- 5
+5	82	+ 69
+6	56	+ 52
+7	<14	+ 14

1 16
+0	39	- 35
+1	28	- 30
+2	126	- 121
+3	25	+ 20
+4	<16	+ 1
+5	43	- 40

2 16
| +0 | <26 | + 19 |

0 17
+1	<25	+ 13
+2	106	+ 83
+3	<24	+ 18
+4	76	- 25
+5	76	+ 61
+6	40	- 50
+7	< 5	- 4

1 17
+0	57	+ 64
+1	27	- 25
+2	40	- 27
+3	40	- 37
+4	<13	- 6
+5	<13	- 10

2 17
| +0 | <25 | - 28 |

0 18
+0	106	- 80
+1	<24	+ 19
+2	<24	- 12
+3	88	+ 86
+4	115	+ 84
+5	76	- 54
+6	<14	- 7

1 18
+0	71	+ 63
+1	<18	- 16
+2	<17	+ 13
+3	46	- 59
+4	40	- 46

2 18
| +0 | <21 | - 17 |

0 19
+1	56	- 56
+2	<23	+ 23
+3	56	+ 48
+4	<18	- 25
+5	<14	- 14

1 19
+0	<17	- 7
+1	<16	+ 10
+2	<15	- 13
+3	37	- 35
+4	<15	+ 22

2 19
| +0 | <17 | + 26 |

0 20
+0	<26	+ 11
+1	59	+ 64
+2	<20	+ 20
+3	<18	+ 12
+4	59	+ 41
+5	< 8	+ 12

1 20
+0	<15	+ 12
+1	53	- 66
+2	<15	+ 11
+3	<15	+ 9

2 20
| +0 | <10 | - 14 |

Table 1 (cont.)

```
  l 0              l 2                l 5                l 9               l 13             l 17
- 2 < 15 + 28    - 1 284 + 347    - 1 302 + 340    - 1   47 -  30    - 1  26 -  15   - 1 < 19 -   3
- 4 130 +  98    - 2 200 - 236    - 2 143 - 133    - 2   98 -  78    - 2  55 +  44   - 2 121 + 135
- 6 163 - 174    - 3  47 -  41    - 3 282 - 291    - 3   98 - 122    - 3 214 + 214   - 3  38 +  29
- 8 203 - 223    - 4  69 +  57    - 4 206 - 196    - 4   52 -  60    - 4  86 -  83   - 4 < 18 -   7
-10 < 25 -  16   - 5 121 + 134    - 5  48 -  42    - 5 < 18 +   0    - 5  95 +  83   - 5 122 -  91
                 - 6  40 +  18    - 6 108 -  99    - 6 < 19 +   5    - 6  82 +  73   - 6 < 17 -   6
  2 0            - 7 158 + 155    - 7  27 +  29    - 7   98 - 107    - 7  63 -  71   - 7 < 15 -   1
- 2 137 - 153    - 8 < 20 -  22   - 8  62 +  64    - 8 104 -  84    - 8 < 16 -  11
- 4 137 - 162    - 9  60 +  47    - 9 123 + 119    - 9 < 17 -  11    - 9 < 16 -   9     l 18
- 6  86 +  45    -10  32 -  29    -10  45 -  22    -10 < 12 -  13                    - 1 < 18 +   1
- 8 < 30 +  23   -11 < 20 -  13   -11 < 17 -  37                       l 14          - 2  32 +   8
-10  42 -  13                                        l 10           - 1  27 -   9   - 3  48 +  32
                   l 3              l 6            - 1 130 + 121    - 2  82 +  88   - 4  93 -  78
  3 0            - 1  48 +  14    - 1 274 + 284    - 2  88 -  63    - 3 114 - 105   - 5  60 -  55
- 2  80 +  45    - 2 163 - 183    - 2  20 -  16    - 3  46 +  32    - 4 < 20 +  33   - 6 < 17 -   8
- 4  41 +  31    - 3 344 + 401    - 3 < 13 +  21    - 4  18 +  15    - 5 152 - 132   - 7 < 15 +  12
- 6  47 -  19    - 4 183 - 224    - 4 176 - 157    - 5 < 18 +  19    - 6  45 +  50
- 8 133 +  58    - 5  77 +  78    - 5  87 +  93    - 6  55 +  50    - 7 < 17 -   8     l 19
-10  57 +  28    - 6 139 + 128    - 6 < 18 +  20    - 7  52 -  34    - 8 < 14 +  23   - 1  45 +  44
                 - 7  72 +  75    - 7  28 +  34    - 8  82 -  50    - 9 < 14 +   4   - 2 < 17 +  35
  4 0            - 8  28 -  19    - 8  62 +  53    - 9 < 15 +   3                    - 3  64 -  54
- 2  50 -  18    - 9  42 -  14    - 9 < 18 +   2   -10  40 -  25      l 15          - 4 < 15 +  20
- 4 < 26 +   0   -10 < 16 +  13   -10  25 -  16                     - 1  28 +  23   - 5  36 +  30
- 6 < 25 +  16   -11  48 +  48    -11 < 17 -  12     l 11          - 2 123 - 104   - 6 < 15 -  16
- 8 107 -  30                                      - 1 133 - 124    - 3  98 -  77
-10 < 14 -   9     l 4              l 7            - 2  23 -  22    - 4  34 -  11     l 20
                 - 1 168 + 165    - 1 140 +  92    - 3  78 +  80    - 5 < 19 -  17   - 1 < 15 +  18
  1 1            - 2 244 + 274    - 2  75 +  44    - 4 189 + 178    - 6  25 -  19   - 2 < 15 -  13
- 2 178 + 266    - 3  35 +  43    - 3  19 -  15    - 5 < 19 -  17    - 7 < 16 -  17   - 3  29 +   9
- 3  40 +  50    - 4  40 -  18    - 4 109 +  68    - 6 < 19 +   5    - 8 < 13 +  11   - 4  35 -  30
- 4  57 +  58    - 5 228 - 207    - 5 142 - 150    - 7  27 +  14                    - 5  15 -   7
- 5 119 + 137    - 6  41 -  43    - 6  88 -  87    - 8  49 +  35      l 16
- 6  72 -  81    - 7 < 18 -  10   - 7  97 + 105    - 9  61 -  60    - 1 < 20 -  14     l 21
- 7  82 +  69    - 8  48 +  37    - 8  28 +  26    -10 < 17 +  11    - 2 < 20 -  16   - 1 < 13 -  18
- 8 185 + 196    - 9  18 +  18    - 9  55 -  42                     - 3  58 -  65   - 2 < 13 +  16
- 9  33 -  10    -10 < 16 +  34   -10  25 +  47      l 12          - 4 < 19 +  11   - 3 < 12 +   5
-10  36 +  29    -11  34 -  36                     - 1 110 - 107    - 5 < 18 +   6   - 4  49 +  46
-11 < 17 +  12                     l 8            - 2 < 18 -  13    - 6  82 -  60
                                 - 1 218 - 178    - 3 < 18 +   0    - 7 < 14 +   2
                                 - 2  40 -  25    - 4 168 + 196    - 8  33 -  18
                                 - 3 203 + 218    - 5 157 + 165
                                 - 4 106 -  85    - 6  28 +  33
                                 - 5 < 17 -   2    - 7  70 +  62
                                 - 6 < 18 +   4    - 8 < 17 +   1
                                 - 7  98 -  79    - 9 < 17 -  17
                                 - 8  74 +  71
                                 - 9  45 -  40
                                 -10 < 13 +   5
```

Results

The final atomic coordinates with their standard deviations are shown in Table 2.

Table 2. Final parameters and their standard deviations

Atom	x_i	y_i	z_i	$\sigma(x_i)$	$\sigma(y_i)$	$\sigma(z_i)$
C_1	0·5760	0·2638	0·3566	0·0039	0·0005	0·0010
C_2	0·5827	0·3852	0·4965	0·0040	0·0005	0·0010
N_1	0·5719	0·1900	0·3807	0·0031	0·0004	0·0008
N_2	0·6107	0·3070	0·4891	0·0032	0·0004	0·0008
N_3	0·5541	0·4260	0·3659	0·0029	0·0004	0·0008
O_1	0·5781	0·2892	0·2294	0·0026	0·0003	0·0007
O_2	0·6058	0·4123	0·6259	0·0027	0·0003	0·0007
O_3	0·2016	0·0440	0·4616	0·0041	0·0005	0·0011
H_1	0·573	0·172	0·485			
H_2	0·583	0·156	0·293			
H_3	0·600	0·274	0·573			
H_4	0·549	0·399	0·267			
H_5	0·500	0·481	0·369			
H_6	0·492	0·060	0·344			
H_7	Not determined					

Thermal parameter: $B = 2.57$ Å^2, $\alpha = 0.087$.

That the accuracy of the x-coordinates was much lower than that of the y- and z-coordinates was to be expected, since most of the F_{hkl}'s have $h < 2$. At the end of the final refinement cycle: $\Sigma F_o = 3875$, $\Sigma F_c = 3788$, and $T_f = \exp(-2.57 \sin^2 \theta / \lambda^2 - 0.087 h^2)$. The final r.m.s. changes of the coordinates were:

$$[\Sigma(\delta x_i)^2]^{\frac{1}{2}} = 0.0029, \quad [\Sigma(\delta y_i)^2]^{\frac{1}{2}} = 0.0006,$$
$$[\Sigma(\delta z_i)^2]^{\frac{1}{2}} = 0.0006 \text{ Å}.$$

Dimensions of biuret molecule

The dimensions of the biuret molecule are shown in

Fig. 5 and listed in Table 3. In this table we have included comparisons with standard bond-lengths for C–N (1·474 Å (Donohue, Lavine & Rollett, 1956)) and for C=O (1·215 Å (Pauling, 1948)) using the significance limits for the t_0-test proposed by Cruickshank & Robertson (1953). The bond-orders were calculated by Pauling's expression (1948),
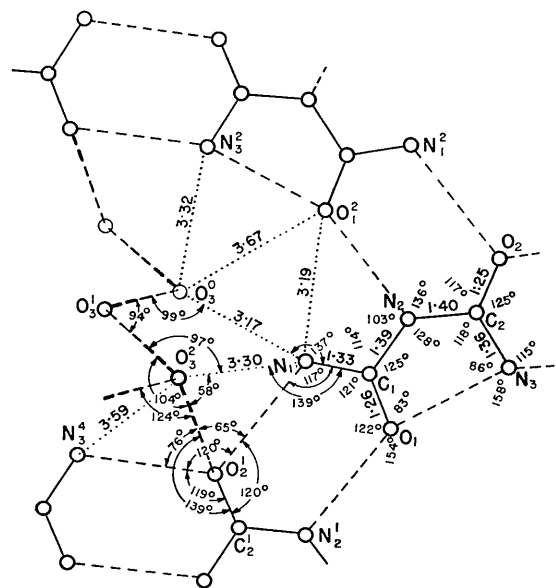

Fig. 5. Projection on (100), showing intra-molecular bond-lengths for biuret molecule, angles between bonds and between some close approach-distances, and environment of water-sites.

Table 3. *Biuret molecule—bond lengths* (l) *and angles* (θ), *with standard deviations* (σ), *differences from standard bonds* $(l-l_0)$, *significance tests for* $(l-l_0)$, *and bond orders*

$$[t_0 = (l-l_0)/\sigma(l)]$$

Bond	l	$\sigma(l)$	Standard bond	$l-l_0$	t_0	Significance	Bond order
C_1-N_1	1.33_2 Å	0.024	C–N	0.142 Å	5.9	Highly sig.	1.42
C_1-N_2	1.39_1	0.024	C–N	0.083	3.5	Highly sig.	1.18
C_2-N_2	1.39_9	0.024	C–N	0.075	3.1	Significant	1.16
C_2-N_3	1.36_1	0.024	C–N	0.113	4.7	Highly sig.	1.28
C_1-O_1	1.25_5	0.023	C=O	0.040	1.8	Not sig.	1.60
C_2-O_2	1.24_6	0.023	C=O	0.031	1.4	Not sig.	1.66

Angle	θ	$\sigma(\theta)$	Angle	θ	$\sigma(\theta)$
$N_1-C_1-N_2$	113.8°	1.6	$N_2-C_2-O_2$	117.2°	1.7
$N_1-C_1-O_1$	120.9	1.7	$N_3-C_2-O_2$	124.9	1.7
$N_2-C_1-O_1$	125.0	1.7	$C_2-N_3 \cdots O_1$	86	—
$C_1-N_2-C_2$	127.9	1.5	$C_1-O_1 \cdots N_3$	83.1	—
$N_2-C_2-N_3$	117.6	1.6			

$$r_x = r_1 - (r_1 - r_2).3x/(2x+1),$$

where x is the double-bond character of a bond length r_x and r_1, r_2, are the lengths of the corresponding pure single and double bonds, respectively.

It is seen that the carbon–oxygen bonds are significantly longer than pure C=O bonds. All the carbon–nitrogen bonds have appreciable double bond character, the two central C–N bonds having about half as much as the terminal ones.

There are no significant deviations from the least-squares planes which can be fitted to the four atoms of each half-molecule; the two NH₂–CO–NH– fragments are inclined at 5° 33′ to each other. This appears to be caused by repulsion between O_1 and N_3, a supposition which is supported by the enlargement of the internal angles of the $O_1C_1N_2C_2N_3$-pentagon above the values from the bond-orders of the corresponding bonds.

From this point of view it is interesting that in the iso-electronic molecule nitro-guanidine (Bryden *et al.*, 1956) the intra-molecular N–H · · · O hydrogen-bond is much shorter (2·57 Å) than is the corresponding bond in biuret (2·76 Å). The angle of the pentagon which is opposite the hydrogen-bond is 118° in nitroguanidine and 127° in biuret. From the double-bond character of the N–O bonds in nitroguanidine it appears that the oxygen atom has a formal charge of 0·6 electrons. In biuret, a similar calculation for the C_1-O_1 bond gives O_1 a formal charge of 0·4 electrons. The differences in the lengths and strengths of the N–H · · · O bonds in these two molecules are thus associated with a difference in the electronegativities of the oxygen atoms.

Other structures with which biuret should be compared are those of oxamide (Ayerst & Duke, 1954) and succinamide (Davies & Pasternak, 1956). All three structures possess planar amide groups *trans* to each other although—in contrast to biuret—oxamide and succinamide possess totally planar molecules.

A survey of bond lengths and bond angles in these and related molecules has recently been published by

Davies & Pasternak (1956). Their tables will not be reproduced here. The following data will suffice to establish the dimensional similarity between biuret, the other two di-amides, and urea.

	Bond lengths			Bond angles		
	C–O	C–N	$\sigma(l)$	$\overset{HN}{\underset{C}{}}$–C–N	$\overset{HN}{\underset{C}{}}$–C–O	N–C–O
Molecule	(Å)	(Å)	(Å)	(°)	(°)	(°)
Oxamide	1.243	1.315	0.004	114.8	119.5	125.7
Succinamide	1.238	1.333	0.002	115.6	122.4	122.0
Urea	1.262	1.335	0.010	118.0	(121.0)	121.0
Biuret						
(1)	1.25_5	1.33_2	0.024	113.8	125.0	120.9
(2)	1.24_6	1.36_1	0.024	117.6	117.2	124.9
Ave.	1.25	1.35		115.7	121.1	122.9

Almost exact agreement with the experimental bond-orders results from a linear combination of the eight valence-bond structures allowed for the biuret molecule, if the uncharged structure is given about triple the weight of those involving charge-separations. (We exclude from consideration a ninth structure involving *two* double-bonds to the central nitrogen-atom, N_2, as this would require a bond-angle of 180°. The impossibility of this structure is the principal reason for the lengthening of the two C–N bonds, C_1-N_2 and C_2-N_2.)

Description of structure

If one were to look at the crystal along a line parallel to the *z*-axis, one would see slightly buckled sheets of biuret molecules lying roughly parallel to (100) at $x = 0.57$ and $x = 0.43$ in adjacent halves of the unit-cell. Within these corrugated layers, the molecules are held together by infinite systems of N–H · · · O hydrogen-bonds. Four of these are quite normal:

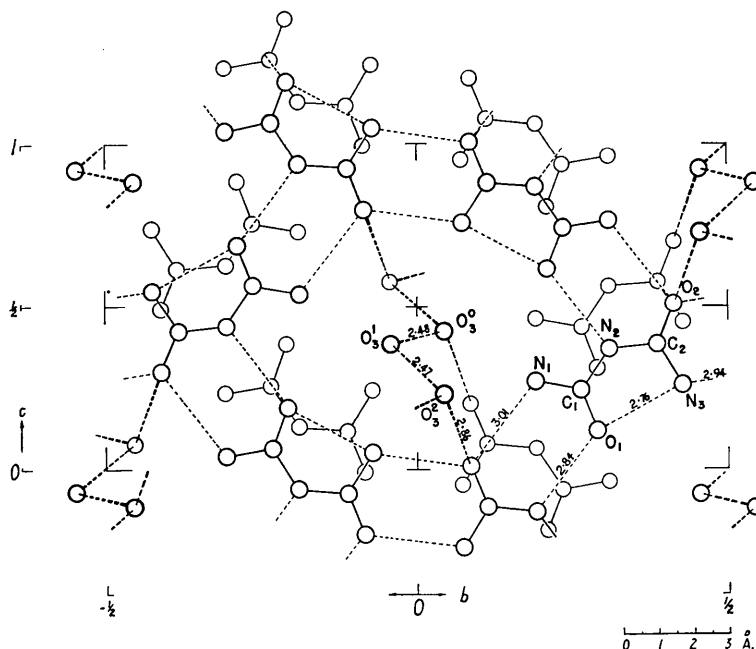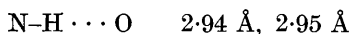|		
N_3–H · · · O_1	2.76 Å	(intra-molecular)
N_2–H · · · O_1^2	2.84 Å	(inter-molecular)
N_1–H · · · O_2^1	3.01 Å	(inter-molecular)
N_3–H · · · O_2^3	2.94 Å	(inter-molecular)

Fig. 6. Normal projection on (100), showing two layers of the crystal. The bottom layer is drawn with light lines.
Broken lines signify hydrogen-bonds, whose lengths are shown.

cf. oxamide

N–H $\cdots$ O   2·94 Å, 2·95 Å

succinamide

N–H $\cdots$ O   2·94 Å

There is one doubtful short contact, $N_1 \cdots O_1^2$ (3·19 Å) which does not lie in a direction — with respect to either atom—favourable for hydrogen-bond formation (see Table 4).

Table 4. *Angles between some inter-atomic vectors*

Subscripts have been used to distinguish the atoms within an asymmetric unit. Super-scripts identify the atoms of different asymmetric units

Angle	θ	Angle	θ
$C_1–N_1 \cdots O_2^1$	117·3°	$N_1 \cdots O_3^1 \cdots N_3^4$	119·9°
$C_1–N_1 \cdots O_3^0$	137·3	$N_3^4 \cdots O_3^1 \cdots O_3^2$	76·2
$C_1–N_1 \cdots O_3^2$	138·6	$N_1 \cdots O_3^1 \cdots O_3^2$	65·1
$C_1–N_1 \cdots O_1^2$	153·4	$O_3^0 \cdots O_3^1 \cdots O_3^2$	94·4
$C_1–O_1 \cdots N_2^1$	121·7	$O_3^1 \cdots O_3^0 \cdots N_1$	98·9
$C_1^2–O_1^2 \cdots N_1$	167·2	$O_3^1 \cdots O_3^2 \cdots N_1$	96·9
$N_3 \cdots O_1 \cdots N_2^1$	154·0	$O_3^1 \cdots O_3^2 \cdots O_1^1$	103·9
$C_2^1–O_2^1 \cdots N_1$	119·9	$N_1 \cdots O_3^2 \cdots O_1^1$	58·1
$C_2^1–O_2^1 \cdots N_3^4$	119·3	$O_3^1 \cdots O_3^2 \cdots O_1^1$	123·7
$C_2–O_2^1 \cdots O_3^2$	139·0	$O_3^1 \cdots O_3^2 \cdots N_1$	165·3
$O_2^1 \cdots N_3^4 \cdots O_1^4$	157·5		
$O_2^1 \cdots N_3^4–C_2^4$	115·4		

Adjacent layers of biuret molecules are held together largely, though not exclusively, by van der Waals forces. This is consistent with the easy cleavage of the crystals parallel to (100). Electrostatic forces probably also play a part: the superposition of two layers, shown in Fig. 6, places the small negative charges on the oxygen atoms almost vertically above/ below the (relatively) positive charges on the nitrogen atoms of the next layer.

Large tunnels run through the crystal in the x-direction (i.e. at about 60° to the hydrogen-bonded sheets of molecules). The 'hollow' part of each tunnel —that part which is further than the average van der Waals radius from the nearest atomic centres—is about 4 Å in diameter.

In these tunnels, connected to the 'walls' by relatively weak hydrogen bonds, are situated the water molecules. Their distances from other atoms are:

$O_3^2 \cdots O_2^1$	2·86 Å		$O_3^0 \cdots N_1$	3·17 Å
$O_3^2 \cdots N$	3·30		$O_3^0 \cdots N_3^2$	3·32
$O_3^2 \cdots N_3^4$	3·59		$O_3^0 \cdots O_1^2$	3·67
$O_3^2 \cdots O_1^2$	5·05		$O_3^0 \cdots O_2^1$	4·10
$O_3^2 \cdots N_3^2$	5·31			

The shortest oxygen–nitrogen contact, $O_3^0 \cdots N_1$, occurs at 137° to the $C_1–N_1$ bond, so that only the oxygen–oxygen contact between O_3^2 and O_2^1 is unequivocally a hydrogen bond. As such it has a length corresponding to a weak bond (Donohue, 1952) in the range usually found for O–H $\cdots$ O bonds (2·75–3·0 Å).

Loosely attached as they are to the biuret structure, the water molecules partially occupy sites along a zig-zag chain, adjacent sites being related by what would be centres of symmetry in an ordered crystal. The alternate site–site distances are equal—

$O_3^0 \cdots H \cdots O_3^1$ $2·47_6$ Å

$O_3^1 \cdots H \cdots O_3^2$ $2·47_3$ $\sigma(l) = 0·029$ Å

but their size places them in the much smaller and less understood class of short $O \cdots O$ approach-distances. Shorter $O \cdots O$ distances than 2·47 Å have been reported, but none of them offers much scope for making comparisons with the present case because they do not involve adjacent water-molecules. The only exception occurs in the crystal of caffeine hydrate (Sutor, 1958) where an apparent water–water distance of 2·27 Å has been reported. In this crystal, too, the water-content is variable and lies below the figure expected for a monohydrate.

There is a further (and possibly related) point on which the present study provides no information. The crystallographic evidence shows that at the time of the diffraction experiment the proportion of water in the crystals was about 0·6 H_2O per biuret molecule. Since no precaution was taken to avoid water-loss during the X-ray exposures, this crystallographic conclusion need not be in conflict either with Rollet & Cohen-Adad's figure of 0·8 H_2O/biuret or with the conclusions drawn from the density-determination during the present work. What is certain is that the agreement was better and that the difference-syntheses were flatter for a 0·6-hydrate than for a 0·8-hydrate, the atomic positions being insignificantly affected by the degree of hydration assumed.

How the water molecules are distributed is, however, not clear. The molecules may randomly occupy three-fifths of the possible sites in each tunnel; three out of five tunnels may be completely filled; or each tunnel may be completely filled for three-fifths of its length. Obviously, this structure and that of caffeine hydrate should be further investigated under controlled conditions of humidity. One of us (H. C. F.) proposes to carry out such a study of biuret hydrate, using neutron-diffraction in an attempt also to locate the undiscovered seventh hydrogen atom.

Biuret hydrate as an 'inclusion compound'

Biuret hydrate can properly be described as an inclusion compound of the 'tunnel' type: (i) It contains variable proportions of one component trapped in cavities of the other; (ii) the framework collapses on removal of all of the included species; and (iii) the continuous component, biuret, crystallizes quite differently in the absence of the other. The only feature which distinguishes biuret hydrate from the usual types of inclusion-compounds is the weak hydrogen-bonding between its components. (For a classification of inclusion compounds see Schlenk (1951)).

It is interesting that even the loosely bonded and labile water molecules in biuret hydrate are so important in stabilising the structure. Their lability suggests that they differ from the normal 'water of crystallization'. One is led to speculate whether other structures in amide chemistry may be stabilized by H_2O–H_2O bridges in a similar way.

Editorial Note:

For a third hydrate structure, similar to biuret hydrate and caffeine hydrate in that there are both variable water content and short water–water distances, see 'The Crystal Structure of Thymine Monohydrate' by R. Gerdil, *Acta Cryst.* **14**, 333 (1961). For a discussion of water positions in these hydrates see *ibid.* and 'On the Arrangement of the Water Molecules in the Crystal Structure of Caffeine' by R. Gerdil & R. E. Marsh, *Acta Cryst.* **13**, 165 (1960).

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