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The Crystal and Molecular Structure of Firefly D(-)-Luciferin

BY DON DENNIS* AND R. H. STANFORD JR

Gates and Crellin Laboratories of Chemistry, † California Institute of Technology, Pasadena, California 91109, U.S.A.

(Received 25 March 1971; accepted 18 January 1973)

The structure of firefly luciferin has been confirmed by X-ray diffraction analysis. The compound, $C_{11}H_8O_3N_2S_2$, forms orthorhombic crystals, space group $P2_12_12_1$, with cell dimensions $a=9.410$, $b=22.956$, and $c=5.370$ Å: there are four molecules in the unit cell. The structure was refined by least-squares calculations to an R index of 0.054 for 841 non-zero reflections collected on a diffractometer with $Co K\alpha$ radiation.

Introduction

The blue-green flash of the firefly has been shown to result from the oxidation of the substrate luciferin to yield as a final product dehydroluciferin (Fig. 1).

* Permanent address: Department of Chemistry, University of Delaware, Newark, Delaware 19711, U.S.A.

† Contribution No. 4230 from the Gates and Crellin Laboratories of Chemistry. This work was supported in part by Research Grant No. GB-6617 from the National Science Foundation and in part from Research Grant No. GM-12121 from the National Institutes of Health, National Institute of General Medical Sciences.

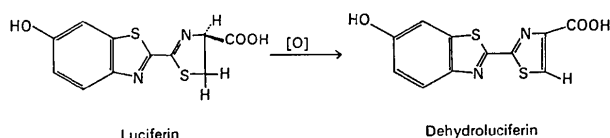
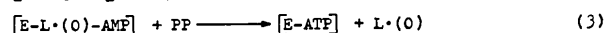
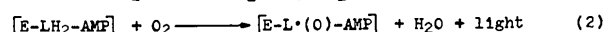
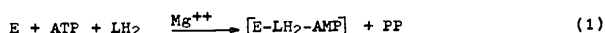


Fig. 1. Oxidation of luciferin.



E = Luciferase ATP = Adenosine triphosphate

LH_2 = Luciferin AMP = Adenosine monophosphate

PP = Pyrophosphate $L \cdot(O)$ = Oxidized luciferin

Fig. 2. Proposed reactions in the enzymatic oxidation of luciferin.

The enzyme luciferase which catalyzes this reaction in fireflies is stereoselective with respect to the asymmetric center and the luminescent event occurs only when the D(-) isomer is oxidized. The chemical structure of D(-)-luciferin was confirmed by a synthesis involving the condensation of 2-cyano-6-hydroxybenzothiazole and D(-)-cysteine. The condensation product employing the L(+)-cysteine was inoperative in the production of the light burst in the enzyme system (White, McCapra, Field & McElroy, 1961; White, McCapra & Field, 1963).

The series of reactions shown in Fig. 2 have been suggested to explain the observed kinetics of luminescence when D(-)-luciferin, adenosine triphosphate, and luciferase are mixed in the presence of Mg^{2+} and O_2 (McElroy & Seliger, 1963). An interesting feature of the proposed reaction (1), Fig. 2, is the formation of the luciferyl-AMP bond which involves the carboxyl group of luciferin and the primary phosphate group of adenosine monophosphate. Information regarding the absolute configuration of the carboxyl group is essential to studies concerned with the positioning of the substrate on the enzyme surface. It should be noted that dehydroluciferin may be the isolable oxidized form of luciferin but it is thought not to be identical with the product $L \cdot(O)$, a bound oxidized form of luciferin associated with an atom of oxygen.

This investigation was undertaken to determine the geometry of the luciferin molecule and, particularly, its absolute configuration.

Experimental

Crystals of synthetic D(-)-luciferin were obtained from E. I. du Pont de Nemours and Company, Wilmington, Delaware. A small, plate-like crystal was chosen for use in the X-ray investigation. A precession camera survey of the crystal exhibited extinctions characteristic of space group $P2_12_12_1$. The unit-cell dimensions obtained from measurements of 2θ angles on a Datex-automated General Electric diffractometer are $a=9.410$ (3), $b=22.956$ (3), $c=5.370$ (1) Å. The density calculated for the crystals is 1.605 g cm^{-3} , assuming four molecules per unit cell. Intensity data were collected on the diffractometer with Fe-filtered $\text{Co K}\alpha$ radiation ($\lambda=1.7902$ Å) to a maximum 2θ angle of 140° ($d_{\text{min}}=0.95$ Å). A θ - 2θ scan technique was employed, background was counted for 30 sec at each end of the scan, and the scan rate was $\frac{1}{2}^\circ$ per min in 2θ . Three check reflections, 200, 101, and 020, were monitored every 50 reflections. The variations in their intensities were well within counting statistics.

All calculations of this analysis were carried out under the CRYM crystallographic computing system on an IBM 360/75 computer. [The CRYM system is based on the CRYRM system for the IBM 7094 computer (Duchamp, 1964).] The data were corrected for Lorentz and polarization effects; but no corrections for absorption were made ($\mu=62 \text{ cm}^{-1}$). Both

the hkl and $h\bar{k}l$ octants of data were collected; these totalled 1438 in number of which 1395 were measurable above background. The averaging of 'equivalent' reflections yielded 841 non-zero reflections. This averaged set of data was used in the determination and

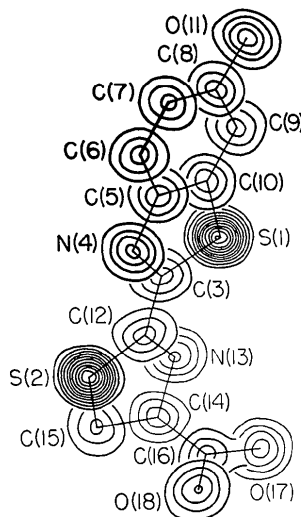


Fig. 3. A composite of sections of a three-dimensional electron density map through each of the heavy-atoms, viewed down the c axis. The lowest contour is at $2 \text{ e} \text{ \AA}^{-3}$. The successive contours are at 4, 6, ... $\text{e} \text{ \AA}^{-3}$.

Table 1. Observed and calculated structure factors

Within each group the columns contain k , $10F_o$, $10F_c$, and $\phi_c(^\circ)$, the phase angle. Reflections marked with an asterisk were assigned zero weight in the final least-squares cycle.

h	k	l	$10F_o$	$10F_c$	$\phi_c(^\circ)$...
0	0	0	15 485 289	-70	3 80	43
2	424	140	19 141 180	-90	3 148	187
4	102	97	16 151 221	90	3 165	221
6	123	140	15 107 187	-90	3 150	187
8	551	99	20 167 187	90	3 55	187
10	275	140	15 107 187	-90	3 150	187
12	48	34	22 57 37	90	3 10	97
14	124	140	15 107 187	-90	3 150	187
16	130	153	0	0	3 11	36
18	130	140	0	0	3 11	116
20	104	160	0	0	3 11	116
22	270	270	0	0	3 11	270
24	270	270	0	0	3 11	270
26	270	270	0	0	3 11	270
28	270	270	0	0	3 11	270
30	270	270	0	0	3 11	270
32	270	270	0	0	3 11	270
34	270	270	0	0	3 11	270
36	270	270	0	0	3 11	270
38	270	270	0	0	3 11	270
40	270	270	0	0	3 11	270
42	270	270	0	0	3 11	270
44	270	270	0	0	3 11	270
46	270	270	0	0	3 11	270
48	270	270	0	0	3 11	270
50	270	270	0	0	3 11	270
52	270	270	0	0	3 11	270
54	270	270	0	0	3 11	270
56	270	270	0	0	3 11	270
58	270	270	0	0	3 11	270
60	270	270	0	0	3 11	270
62	270	270	0	0	3 11	270
64	270	270	0	0	3 11	270
66	270	270	0	0	3 11	270
68	270	270	0	0	3 11	270
70	270	270	0	0	3 11	270
72	270	270	0	0	3 11	270
74	270	270	0	0	3 11	270
76	270	270	0	0	3 11	270
78	270	270	0	0	3 11	270
80	270	270	0	0	3 11	270
82	270	270	0	0	3 11	270
84	270	270	0	0	3 11	270
86	270	270	0	0	3 11	270
88	270	270	0	0	3 11	270
90	270	270	0	0	3 11	270
92	270	270	0	0	3 11	270
94	270	270	0	0	3 11	270
96	270	270	0	0	3 11	270
98	270	270	0	0	3 11	270
100	270	270	0	0	3 11	270
102	270	270	0	0	3 11	270
104	270	270	0	0	3 11	270
106	270	270	0	0	3 11	270
108	270	270	0	0	3 11	270
110	270	270	0	0	3 11	270
112	270	270	0	0	3 11	270
114	270	270	0	0	3 11	270
116	270	270	0	0	3 11	270
118	270	270	0	0	3 11	270
120	270	270	0	0	3 11	270
122	270	270	0	0	3 11	270
124	270	270	0	0	3 11	270
126	270	270	0	0	3 11	270
128	270	270	0	0	3 11	270
130	270	270	0	0	3 11	270
132	270	270	0	0	3 11	270
134	270	270	0	0	3 11	270
136	270	270	0	0	3 11	270
138	270	270	0	0	3 11	270
140	270	270	0	0	3 11	270
142	270	270	0	0	3 11	270
144	270	270	0	0	3 11	270
146	270	270	0	0	3 11	270
148	270	270	0	0	3 11	270
150	270	270	0	0	3 11	270
152	270	270	0	0	3 11	270
154	270	270	0	0	3 11	270
156	270	270	0	0	3 11	270
158	270	270	0	0	3 11	270
160	270	270	0	0	3 11	270
162	270	270	0	0	3 11	270
164	270	270	0	0	3 11	270
166	270	270	0	0	3 11	270
168	270	270	0	0	3 11	270
170	270	270	0	0	3 11	270
172	270	270	0	0	3 11	270
174	270	270	0	0	3 11	270
176	270	270	0	0	3 11	270
178	270	270	0	0	3 11	270
180	270	270	0	0	3 11	270
182	270	270	0	0	3 11	270
184	270	270	0	0	3 11	270
186	270	270	0	0	3 11	270
188	270	270	0	0	3 11	270
190	270	270	0	0	3 11	270
192	270	270	0	0	3 11	270
194	270	270	0	0	3 11	270
196	270	270	0	0	3 11	270
198	270	270	0	0	3 11	270
200	270	270	0	0	3 11	270
202	270	270	0	0	3 11	270
204	270	270	0	0	3 11	270
206	270	270	0	0	3 11	270
208	270	270	0	0	3 11	270
210	270	270	0	0	3 11	270
212	270	270	0	0	3 11	270
214	270	270	0	0	3 11	270
216	270	270	0	0	3 11	270
218	270	270	0	0	3 11	270
220	270	270	0	0	3 11	270
222	270	270	0	0	3 11	270
224	270	270	0	0	3 11	270
226	270	270	0	0	3 11	270
228	270	270	0	0	3 11	270
230	270	270	0	0	3 11	270
232	270	270	0	0	3 11	270
234	270	270	0	0	3 11	270
236	270	270	0	0	3 11	270
238	270	270	0	0	3 11	270
240	270	270	0	0	3 11	270
242	270	270	0	0	3 11	270
244	270	270	0	0	3 11	270
246	270	270	0	0	3 11	270
248	270	270	0	0	3 11	270
250	270	270	0	0	3 11	270
252	270	270	0	0	3 11	270
254	270	270	0	0	3 11	270
256	270	270	0	0	3 11	270
258	270	270	0	0	3 11	270
260	270	270	0	0	3 11	270
262	270	270	0	0	3 11	270
264	270	270	0	0	3 11	270
266	270	270	0	0	3 11	270
268	270	270	0	0	3 11	270
270	270	270	0	0	3 11	270

refinement of the structure. The unaveraged data were used only to determine the correct configuration of the molecule. The standard deviations of the intensities were calculated on the basis of the following equation:

$$\sigma^2(I) = S + (B_1 + B_2)\alpha^2 + (dS)^2$$

where S is the scan count, B_1 and B_2 are the background counts, d is an empirical constant set equal to 0.02, and α is the ratio of scan time to total background

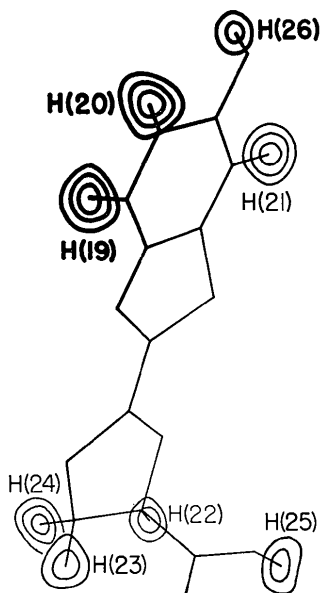


Fig. 4. A composite of sections of a three-dimensional difference Fourier map through each of the hydrogen atoms, viewed down the c axis. The lowest contour is at $0.2 \text{ e } \text{Å}^{-3}$. The successive contours are at $0.3, 0.4, \dots \text{ e } \text{Å}^{-3}$.

time. Finally, the data were placed on an approximate absolute scale by Wilson statistics (1942).

Determination and refinement of the structure

The positions of the sulfur atoms were determined from a three-dimensional Patterson map. A sequence of structure factor, Fourier and difference Fourier calculations led to the complete elucidation of the structure. Initially some difficulties in the interpretation of these maps were encountered because the two sulfur atoms chosen and assumed to be in the same molecule were actually in different molecules. This incorrect assumption resulted from the fact that these two atoms have an intermolecular contact of only 3.73 Å , which lies in the region between the expected distances for *cis*- and *trans*-related sulfur atoms. After the resolution of this problem, the solution of the structure proceeded smoothly.

The structure was refined by full-matrix least-squares techniques. The quantity minimized in the least-squares calculation was $\sum w(F_o^2 - F_c^2)^2$, the weights being taken equal to $1/\sigma^2(F_o^2)$. The atomic scattering factors for C, N, O, and S were taken from *International Tables for X-ray Crystallography* (1962). The

Table 2. Comparison of the stereoisomers

	D(-)	L(+)
$R = \frac{\sum F_o - F_c }{\sum F_o }$	7.0%	8.5%
$R' = \frac{\sum w(F_o ^2 - F_c ^2)^2}{\sum w F_o ^4}$	1.5	2.3
$\left(\frac{\sum w(F_o ^2 - F_c ^2)^2}{m-s} \right)^{1/2}$	2.6	3.2

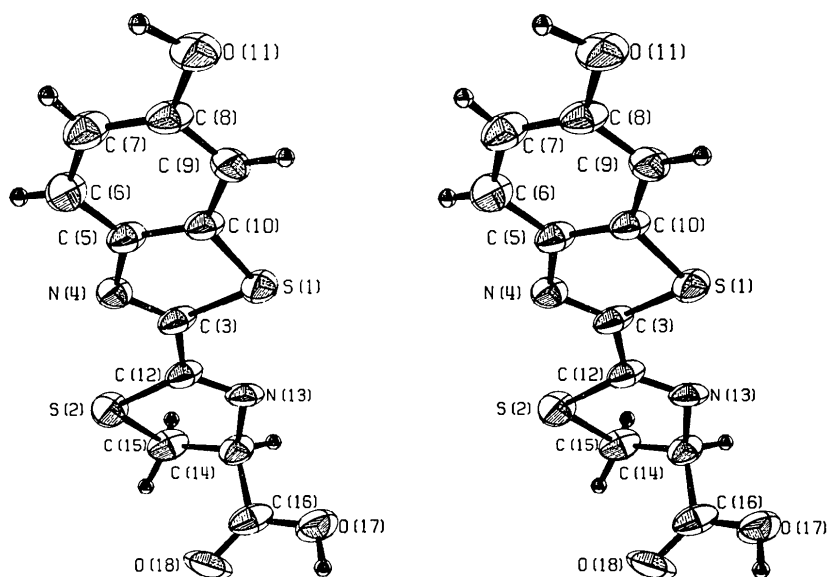


Fig. 5. Stereoscopic view of the luciferin molecule.

Table 3. *Heavy-atom parameters and their standard deviations*

The values have been multiplied by 10^4 . The temperature factor is in the form

$$T = \exp [-(b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl)].$$

	<i>x</i>	<i>y</i>	<i>z</i>	<i>b</i> ₁₁	<i>b</i> ₂₂	<i>b</i> ₃₃	<i>b</i> ₁₂	<i>b</i> ₁₃	<i>b</i> ₂₃
S(1)	5415 (2)	8822 (1)	7765 (5)	102 (2)	10 (0)	471 (13)	-10 (2)	101 (10)	-27 (4)
S(2)	2261 (2)	7458 (1)	9446 (5)	105 (3)	14 (0)	512 (12)	-21 (2)	70 (11)	-17 (4)
C(3)	4002 (7)	8451 (3)	9090 (17)	71 (9)	9 (1)	501 (46)	1 (5)	10 (38)	-28 (14)
N(4)	3363 (6)	8709 (2)	10919 (14)	77 (7)	11 (1)	415 (33)	-12 (5)	43 (30)	-1 (12)
C(5)	3996 (7)	9247 (3)	11374 (16)	73 (8)	10 (1)	443 (47)	-0 (6)	13 (37)	-17 (14)
C(6)	3571 (8)	9635 (3)	13224 (18)	86 (10)	16 (2)	510 (54)	-9 (7)	75 (41)	-9 (15)
C(7)	4292 (8)	10155 (3)	13449 (18)	96 (10)	14 (2)	504 (54)	-5 (7)	17 (40)	-48 (15)
C(8)	5436 (7)	10282 (3)	11932 (17)	83 (9)	10 (1)	508 (47)	-6 (6)	-37 (41)	-24 (14)
C(9)	5878 (8)	9908 (3)	10094 (18)	86 (9)	11 (1)	503 (53)	-13 (6)	92 (39)	18 (14)
C(10)	5136 (7)	9381 (2)	9870 (16)	80 (9)	9 (1)	425 (45)	0 (6)	-2 (35)	-14 (12)
C(11)	6190 (5)	10792 (2)	12177 (12)	130 (7)	12 (1)	601 (36)	-23 (4)	69 (31)	-48 (10)
O(12)	3626 (7)	7871 (3)	8100 (17)	76 (9)	9 (1)	460 (44)	4 (6)	0 (38)	-17 (14)
N(13)	4332 (6)	7662 (2)	6286 (13)	103 (8)	7 (1)	480 (37)	2 (5)	-8 (33)	8 (10)
C(14)	3881 (8)	7052 (3)	5785 (19)	100 (9)	11 (1)	414 (44)	-4 (6)	19 (40)	-34 (15)
C(15)	2420 (8)	6962 (3)	6836 (19)	103 (11)	13 (1)	490 (50)	-6 (7)	-93 (38)	-43 (15)
C(16)	5007 (8)	6656 (3)	6961 (20)	90 (10)	12 (2)	555 (55)	9 (7)	-101 (42)	-40 (16)
O(17)	6273 (5)	6717 (2)	5960 (13)	93 (7)	20 (1)	563 (32)	26 (5)	-45 (27)	22 (12)
O(18)	4783 (6)	6327 (2)	8653 (13)	173 (9)	19 (1)	670 (41)	9 (6)	-31 (35)	144 (12)

atomic scattering factor for H is that given by Stewart, Davidson & Simpson (1965).

After several cycles of refinement of the coordinates and anisotropic temperature factors of the non-hydrogen atoms, the positions of the hydrogen atoms bonded to carbon atoms were calculated. These positional coordinates were then included in the least-squares calculations. The positions of the two remaining hydroxyl hydrogens were determined from a difference Fourier. The coordinates for the hydroxyl hydrogens and the isotropic-temperature parameters of 4.5 \AA^2 , assigned to all of the hydrogen atoms, were not refined. In the final cycles of refinement a secondary extinction factor was included; the expression used is $F_{\text{corrected}}^2 = (F_{\text{cal}})^2 / [1 + g\beta(F_{\text{cal}})^2]$ (Larson, 1967). The final value obtained for the factor *g* is $9.6 (\pm 1.3) \times 10^{-6}$. The final *R* index, $\sum ||F_o| - |F_c|| / \sum |F_o|$, is 0.054; the weighted *R* index, $\sum w(F_o^2 - F_c^2)^2 / \sum wF_o^4$, is 0.007; the 'goodness-of-fit', $[\sum w(F_o^2 - F_c^2)^2 / (m - s)]^{1/2}$ (where *m* is the number of observations and *s* is the number of parameters being refined), is 1.85. The observed and calculated structure factors, *F*_o and *F*_c, and the calculated phase angles are listed in Table 1.

Before the final cycles of least squares were cal-

Table 4. *Hydrogen atom parameters and their standard deviations*

The values have been multiplied by 10^3 . Atoms without indicated standard deviations were not refined. All hydrogen atoms were assigned an isotropic temperature factor of 4.5 \AA^2 .

	<i>x</i>	<i>y</i>	<i>z</i>
H(19)	380 (7)	956 (3)	1402 (15)
H(20)	407 (7)	1042 (3)	1461 (15)
H(21)	668 (6)	999 (3)	933 (15)
H(22)	392 (8)	700 (3)	387 (14)
H(23)	218 (7)	657 (3)	756 (15)
H(24)	176 (7)	704 (3)	558 (16)
H(25)	706	656	711
H(26)	582	1100	1376

culated, structure factors were calculated for the two stereoisomers using all 1395 reflections, both *hkl* and *h $\bar{k}l$* . On the basis of the results of these computations, shown in Table 2, the correct isomer could be easily chosen. The anomalous dispersion corrections for S, $\Delta f' = 0.33$ and $\Delta f'' = 0.75$, were derived by interpolation of the table of Cromer (1965).

The refined atomic parameters for the non-hydrogen atoms are given in Table 3 and for the hydrogen atoms in Table 4. The shifts calculated for the parameters in the final cycle of least squares were all less than one-tenth of the standard deviation.

Discussion of the structure

A composite of sections of a three-dimensional electron density map through the atoms of the molecule is shown in Fig. 3. Fig. 4 is a composite of sections of a three-dimensional difference Fourier map through the hydrogen atoms. A stereoscopic view (Johnson, 1965) showing the structure of the molecule and the thermal ellipsoids for the atoms is given in Fig. 5. Another

Table 5. *Bond distances and angles involving hydrogen atoms*

C(6)—H(19)	0.86 \AA	C(5)—C(6)—H(19)	118°
C(7)—H(20)	0.90	C(7)—C(6)—H(19)	123
C(9)—H(21)	0.90	C(6)—C(7)—H(20)	123
C(14)—H(22)	1.04	C(8)—C(7)—H(20)	116
C(15)—H(23)	1.01	C(8)—C(9)—H(21)	119
C(15)—H(24)	0.93	C(10)—C(9)—H(21)	123
O(17)—H(25)	1.03	N(13)—C(14)—H(22)	106
O(11)—H(26)	1.03	C(15)—C(14)—H(22)	113
		C(16)—C(14)—H(22)	109
		S(2)—C(15)—H(23)	104
		C(14)—C(15)—H(23)	119
		S(2)—C(15)—H(24)	113
		C(14)—C(15)—H(24)	108
		H(23)—C(15)—H(24)	107
		C(16)—O(17)—H(25)	112
		C(8)—O(11)—H(26)	107

stereoscopic view showing the packing of the molecules in the unit cell is shown in Fig. 6.

The bond lengths and angles between the non-hydrogen atoms are shown in Fig. 7. Based on the standard deviations of the coordinates, the estimated standard deviation in the C-S bonds is 0.007 Å and in bonds involving C, N, and O is 0.01 Å. The estimated standard deviation in the bond angles at the sulfur atoms is 0.3° and in bond angles involving C, N, and O is 0.6°. Bond distances and angles involving H-atoms are given in Table 5. The estimated standard deviations in the bond lengths and bond angles involving H atoms are 0.07 Å and 4°.

The benzothiazole system is planar within experimental error. The least-squares plane through the nine atoms of this group is given by the equation:

$$-0.6241X + 0.4343Y - 0.6495Z = 2.294$$

where the coordinates are in ångströms. The deviations of the atoms from this plane are:

S(1)	0.0045 Å	C(3)	0.0023 Å
N(4)	-0.0027	C(5)	0.0027
C(6)	-0.0055	C(7)	0.0108
C(8)	-0.0055	C(9)	0.0023
C(10)	-0.0089.		

The bond angles in the benzene ring, C(5) through C(10), are significantly distorted from an expected value of 120°. The group S(1)-C(3)-C(12)-S(2) exhibits a torsional angle of -176.2°.

The C-H bonds of the benzene and thiazoline rings seem to fall naturally into two groups. The average bond distance, C-H, for the benzene ring is 0.89 Å and for the thiazoline ring is 0.99 Å. Based upon the χ^2 distribution and the estimated standard deviation in the C-H bond lengths of 0.07 Å, the probability that these six bonds are in fact identical is 30%.

Both of the hydroxyl hydrogen atoms are involved in hydrogen bonding. The distances and angles for these intermolecular interactions are:

O(11)···O'(18)	2.71 Å
O(17)···N'(4)	2.76
C(8)-O(11)···O'(18)	107°
C(16)-O(17)···N'(4)	111

As was pointed out above, there is also a short intermolecular S(1)···S'(2) contact of 3.73 Å.

An abstract of this paper was submitted on November 13, 1970 for the winter meeting of the American Crystallographic Association in Columbia, South Carolina (Stanford & Dennis, 1971). After this meeting a brief report of a parallel investigation on the structure of luciferin was published by another group (Blank, Pletcher & Sax, 1971). This team solved the structure by direct methods.

The major difference between these two analyses lies in the measurement of the unit-cell dimensions. The dimensions given by Blank, Pletcher & Sax (1971) are $a=9.248$, $b=22.970$, and $c=5.331$ Å. Although no estimate of the errors in these values is given by the

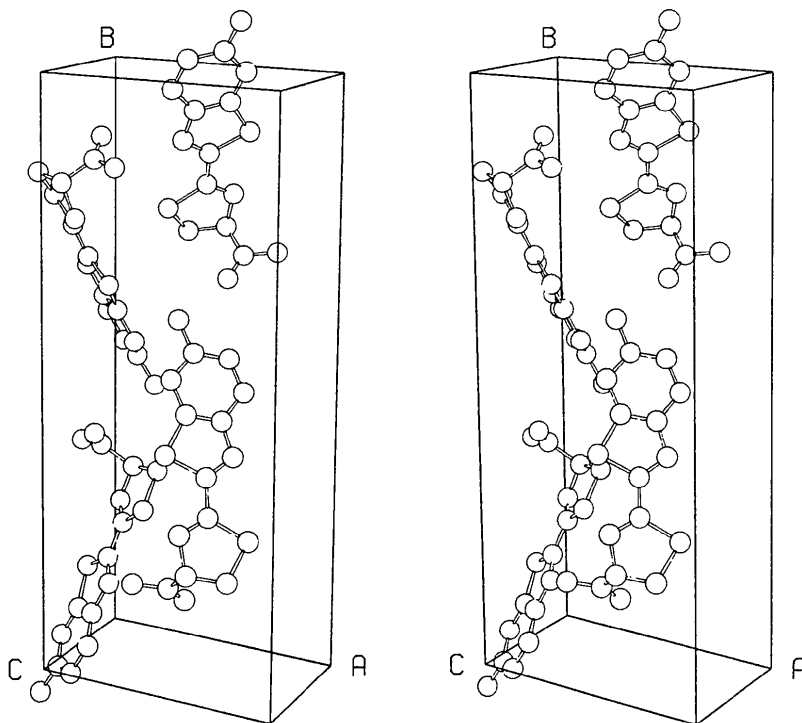


Fig. 6. A stereoscopic view showing the packing of the luciferin molecules in the unit cell.

authors, it is clear that their results differ significantly from those of this paper.

In spite of this discrepancy, information about the errors in the positional coordinates can be obtained from a half-normal probability plot analysis (Abrahams & Keve, 1971). In Fig. 8 the ordered, experimental values of δp are plotted against the expected values of δp (Hamilton & Abrahams, 1972). The quantity δp is the ratio Δ/σ , where Δ is the difference between a corresponding pair of coordinates from the two structure analyses and σ is the standard deviation of this difference, based on the standard deviations of the coordinates derived from the least-squares refinements.

The points of Fig. 8 do not fall along a straight line, indicating some systematic error in one or both of the analyses. The slope of the line given in Fig. 8 is 2.0, showing that the standard deviations of the coordinates from both structure determinations have, on the average, been underestimated by a factor of 2.0.

The authors gratefully acknowledge the assistance of Miss Lillian Casler in the preparation of drawings

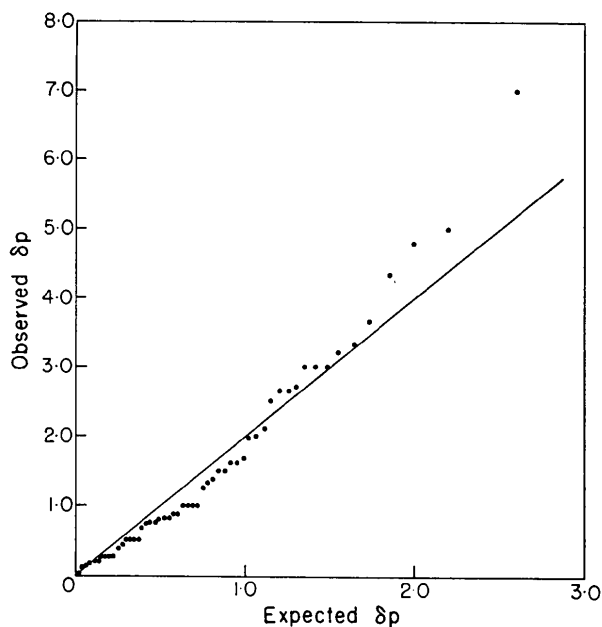


Fig. 8. Half-normal probability plot for the 54 position coordinates refined in the two independent structure analyses.

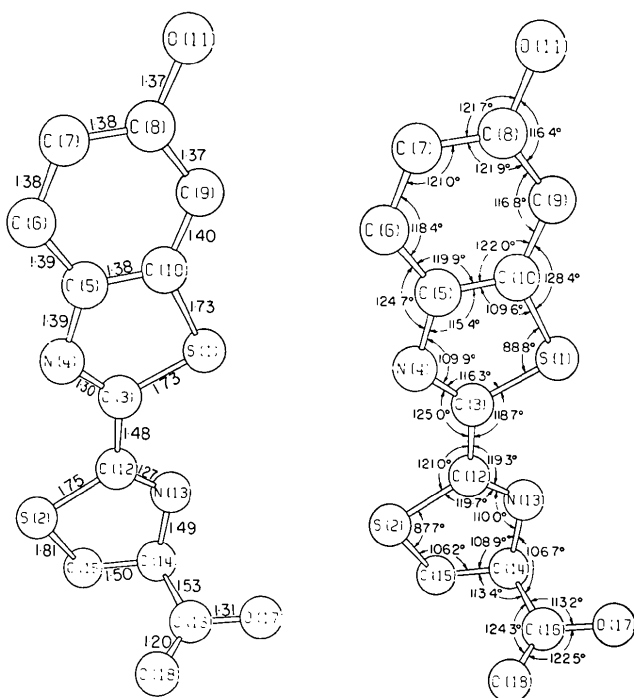


Fig. 7. Bond distances and angles for the heavy atoms of luciferin.

and of Miss Allison Kimball in the preparation of the manuscript.

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