THE MOLECULAR STRUCTURE OF FIREFLY D-(-)-LUCIFERIN: A SINGLE CRYSTAL X-RAY ANALYSIS

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SUMMARY

Crystals of luciferin in space group $P2_12_12_1$ gave cell parameters of a = 9.248, b = 22.970, c = 5.331 Å, D_c = 1.644 gm/cm^3 with 4 molecules per unit cell. The structure and absolute configuration were confirmed to be identical to that established by chemical procedures. Resonance effects of the 6'-phenolic group are distinctly evident. The rings are oriented with the N=C-C=N system trans planar. The carboxyl and thiazoline sulfur are positioned to the same side of the thiazoline ring. The non-bonded intramolecular contacts are normal.

INTRODUCTION

In firefly luciferin the rings are subject to unknown conformational restrictions. In order to establish if any interactions occurred that might be a consideration in the mechanism of bioluminescence, we determined the structure of luciferin by single crystal x-ray crystallographic techniques. The results provide a measure of luciferin's conformational restraints.

MATERIALS AND METHODS

Synthetic luciferin was obtained through E. I. du Pont de Nemours and Company, Wilmington, Delaware. Crystals grown from methyl alcohol showed extinctions for h00, h = 2n+1; 0k0, k = 2n+1; and 00ℓ, ℓ = 2n+1 consistent with the unambiguous assignment of space group $P2_12_12_1$. The cell dimensions are a = 9.248, b = 22.970, c = 5.331 Å, D_c = 1.644 gm/cm^3, D_o = 1.624 gm/cm^3 (by flotation) with 4 molecules per unit cell. Intensity data were collected by (θ/2θ) scans on a Picker FACS I automatic diffractometer using CuKα radiation. The structure was solved by the symbolic addition procedure (1). Phased E values from the tangent formula refinement (2) of an expanded set of phases for E ≥ 1.54 gave an electron density map which contained all the non-hydrogen atomic positions. Anisotropic least squares refinement (3) of the structure (hydrogens isotropic) gave a final residual $R = \Sigma \big| |F_o| - |F_c| \big| / \Sigma |F_o|$ of 0.034. The position of the carboxyl hydrogen was not evident in the difference Fourier synthesis. Positional and thermal parameters are given in Table I. The authors can make available a list of observed and calculated structure factors upon request.

RESULTS AND DISCUSSION

The molecular configuration and conformation of luciferin together with the interatomic bond distances and angles are shown in Fig. 1. Estimated standard deviations for C-S distances and C-S-C angles are ±0.005 Å and ± 0.2°, and for other bonds and angles excluding hydrogen atoms ±0.006 Å and ±0.4°. Bonds S_1'-C_2' and N_3'-C_9' are considerably

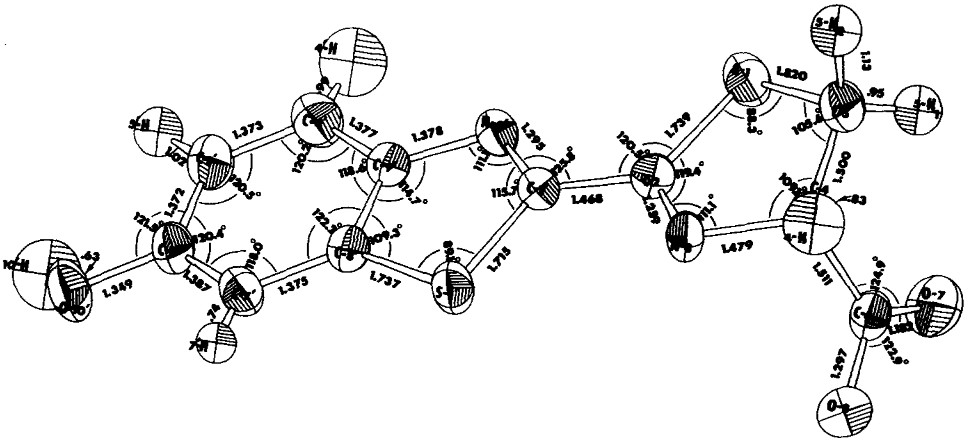


Fig. 1. Projection of the structure of luciferin down [c].

shorter than reported for 2-substituted benzothiazoles (4, 5). Similarly the bond S_1-C_2 is shorter relative to a 2-substituted thiazoline (6). It was inferred that this shortening is in part attributable to electron delocalization into sulfur. Structural formulas I and II, and in the thiazoline system, III through V, are suggested as important resonance structures. Resonance

Table I

Atomic positional and thermal parameters[a] of luciferin with their estimated standard deviations[b]

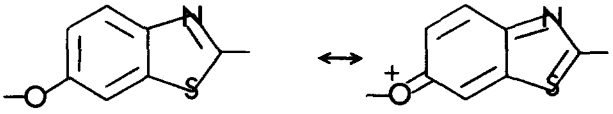
Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
S-1	0.2313(1)	0.2544(1)	0.0550(2)	92(1)	14(0)	375(5)	9(0)	-27(2)	-8(1)
O-7	0.4832(5)	0.3664(2)	0.1383(9)	131(5)	18(1)	590(21)	-4(2)	10(9)	57(4)
O-8	0.6332(4)	0.3280(2)	0.4065(8)	89(4)	17(1)	389(15)	-4(1)	-1(7)	-1(3)
S-1'	0.5455(1)	0.1179(0)	0.2228(2)	95(1)	10(0)	360(4)	5(0)	-45(2)	-12(1)
O-10'	0.6171(5)	-0.0793(2)	-0.2205(9)	127(5)	13(1)	401(16)	14(1)	-39(9)	-20(3)
C-2	0.3682(5)	0.2131(2)	0.1905(9)	70(4)	11(1)	311(16)	0(2)	21(8)	0(3)
N-3	0.4391(4)	0.2338(1)	0.3713(8)	88(4)	8(1)	352(15)	2(1)	20(7)	3(3)
C-4	0.3952(5)	0.2945(2)	0.4246(10)	99(5)	8(1)	289(18)	1(2)	27(9)	-1(3)
C-5	0.2477(5)	0.3050(2)	0.3163(10)	88(5)	12(1)	369(19)	3(2)	35(9)	-10(3)
C-6	0.5062(5)	0.3342(2)	0.3063(9)	88(5)	9(1)	308(17)	-2(2)	15(9)	-4(3)
C-2'	0.4042(4)	0.1553(2)	0.0906(10)	62(4)	10(1)	371(18)	1(2)	8(9)	-4(3)
N-3'	0.3407(4)	0.1289(1)	-0.0958(8)	71(4)	11(1)	357(15)	4(1)	-13(7)	-9(3)
C-4'	0.3572(5)	0.0365(2)	-0.3213(11)	95(5)	16(1)	405(20)	5(2)	-21(11)	-13(4)
C-5'	0.4293(5)	-0.0153(2)	-0.3485(11)	104(5)	12(1)	397(20)	1(2)	-11(10)	-19(4)
C-6'	0.5446(5)	-0.0286(2)	-0.1970(11)	92(5)	10(1)	378(18)	3(2)	30(10)	-10(3)
C-7'	0.5877(5)	0.0095(2)	-0.0097(11)	81(5)	12(1)	377(20)	7(2)	-42(9)	-10(4)
C-8'	0.5148(5)	0.0615(2)	0.0122(9)	76(4)	10(1)	294(16)	-1(2)	1(8)	-7(3)
C-9'	0.4008(5)	0.0760(2)	-0.1423(9)	67(4)	11(1)	343(17)	0(2)	-3(8)	-7(3)
4-H	0.401(7)	0.299(3)	0.578(14)	4.4(1.5)					
5-H_1	0.239(5)	0.344(2)	0.256(11)	2.8(1.0)					
5-H_2	0.155(6)	0.297(2)	0.449(11)	3.0(1.0)					
4'-H	0.278(7)	0.051(2)	-0.433(13)	5.4(1.4)					
5'-H	0.400(5)	-0.044(2)	-0.485(9)	2.8(0.9)					
7'-H	0.658(6)	0.000(2)	0.049(10)	1.9(0.9)					
10'-H	0.601(8)	-0.092(3)	-0.320(14)	5.8(1.9)					

[a] The form of the temperature factor expression used was $\exp[-(h^2\beta_{11} + k^2\beta_{22} + \ell^2\beta_{33} + 2hk\beta_{12} + 2h\ell\beta_{13} + 2k\ell\beta_2$

All β_{ij} have been multiplied by 10^4 except the isotropic parameters of the hydrogen atoms.

[b] Standard deviations of the least significant figures are in parentheses.

form III is stabilized by the electron donating tendencies of the benzothiazole system, consistent with the observed shortening of the C_2-C_2' bond.



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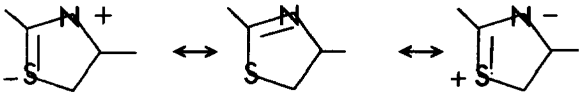

III IV V

The preferred conformation of luciferin in the solid state is with the rings oriented such that the N=C-C=N system is maximally extended. Although resonance through the bridging C-C bond is insufficient alone to restrict the rings to this conformation (7), steric and non-bonding interactions between nitrogen and sulfur (N_3---S_1', 2.948 Å and N_3'---S_1, 3.140 Å) contribute toward maintenance of the trans planar conformation.

Atoms S_1, C_2, N_3, S_1', C_2' and N_3' are nearly coplanar. Displacement values from an unweighted least squares plane calculation based on the planar arrangement of atoms 2, 3, 2' and 3', are: -0.037, 0.004, -0.004, -0.029, 0.005, and -0.004 Å respectively. A similar calculation for the benzothiazole ring atoms, S_1' through C_9', showed it to be nearly planar; the maximum out of plane deviation being 0.017 Å. Distortion of the benzene portion from an ideal hexagon, Fig. 1, has been observed previously (4).

The thiazoline ring is puckered with C_4, -0.095 Å and C_5, 0.340 Å out of the plane of S_1, C_2, and N_3. Substituents on C_5 and C_4 are partially eclipsed. The torsion angle for S_1-C_5-C_4-N_3 is -26.76°. The carboxyl group is in the usual synplanar conformation (8), the torsion angle C_5-C_4-C_6-O_7 being -4.97°.

Prior to the final refinement the (L) and (D) enantiomorphs were refined by full matrix least squares to convergence using the anomalous dispersion contributions of sulfur and oxygen. The residual index for the (D) enantiomer was 0.040 and that of the (L), 0.050. In order to strengthen the indication of correct enantiomorph (9) a number of reflections sensitive to the anomalous dispersion effects were collected in symmetry related pairs. The calculated and observed intensity differences for the correct enantiomorph are given in Table II and establish the configuration as shown in Fig. 1 (ref. 10).

Table II

Comparison of enantiomer sensitive reflections.

$$2[(F^2_{hk\ell} + F^2_{\bar{h}\bar{k}\ell}) - (F^2_{\bar{h}\bar{k}\bar{\ell}} + F^2_{hk\bar{\ell}})] / \Sigma F^2_{hk\ell}$$

h	k	ℓ	Obs.	Calc.	
				D-Enan.	L-Enan.
5	4	2	-0.19	-0.36	0.34
8	10	1	-0.07	-0.25	0.26
2	4	3	-0.11	-0.26	0.24
6	15	1	0.14	0.32	-0.33
2	6	1	-0.24	-0.27	0.28
1	11	1	0.25	0.19	-0.19
1	16	2	0.15	0.21	-0.21
1	15	3	0.26	0.31	-0.32

Any significance of the subtle structural features of luciferin to its biochemical properties cannot be resolved without further study.

ACKNOWLEDGEMENTS

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Erratum

Volume 41, No. 2 (1970), in the Communication, "Cytokinin Activity in $tRNA^{Phe}$," by S. M. Hecht, R. M. Bock, N. J. Leonard, R. Y. Schmitz, and F. Scoog, pages 435-440, Page 437 - RESULTS AND DISCUSSION - the first sentence should read:

> "The fluorescent base of tRNA (2.0 g) derived[4,5] from wheat germ was used to record a time-averaged 100 MNz nmr spectrum in $CDCl_3$ at -20^{o}".