Tetrahedron Letters, No. 5, pp. 4-9, 1959. Pergamon Press Ltd. Printed in Great Britain.

THE STRUCTURE OF CYPRIDINA LUCIFERIN

Yoshimasa Hirata, Osamu Shimomura and Shoji Eguchi
Nagoya University, Nagoya, Japan

(Received 22 April 1959)

CYPRIDINA luciferin was obtained crystalline, and preliminary results on the constitution of luciferin using the crystalline material have already been reported.[1]

Molecular Weight and Molecular Formula of Cypridina Luciferin

The molecular weight of crystalline luciferin was estimated to be 470 by various methods; Barger (357), oxidative titration with ferricyanide (467), the U. V. absorption of hydroluciferin (478), hydrogenation of luciferin (498), and analyses of chlorine and nitrogen.

The percentage analysis is as follows:

	C	H	N	O	Cl	Ash	M. W.
Found	53.2	6.38	18.86	6.7	16.2	0	
Calcd. for $C_{21}H_{28}O_2N_6 \cdot 2HCl$	53.7	6.39	17.91	6.8	15.1	0	469

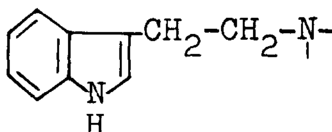
The results from hydrolysis experiments and pKa' measurements of luciferin dihydrochloride were also taken into consideration in deducing the molecular formula of $C_{21}H_{28}O_2N_6 \cdot 2HCl$.

[1] O. Shimomura, T. Goto and Y. Hirata, Bull. Chem. Soc. Japan 30, 929 (1957).

Deduction of the Structure of Hydroluciferin

When hydrogenated with Adams' platinum oxide catalyst, luciferin absorbed 3 moles of hydrogen and was converted into a compound which showed an U. V. absorption spectrum suggesting the presence of a β-indolethylamino group. A positive Ehrlich reaction showed that the α-position of the indole nucleus is free and hydrolysis of hydroluciferin afforded tryptamine.

Hydroluciferin thus contains the grouping



The Sakaguchi reaction is positive for hydroluciferin as well as for luciferin and oxyluciferins, and this shows the presence of a monosubstituted guanidino group which in turn is confirmed by the isolation of γ-guanidinobutyric acid by acid hydrolysis in the presence of oxygen(air). γ-Guanidinobutyric acid was produced only when the hydrolysis was conducted under the presence of oxygen. Thus the γ-guanidinobutyric acid moiety is not contained in a peptide linkage; it is present in a more reduced state.

Isoleucine was also obtained by hydrolysis. Accordingly hydroluciferin is composed of tryptamine, γ-guanidinobutyric acid and isoleucine. This accounts for 21 carbon atoms and 6 nitrogen atoms.

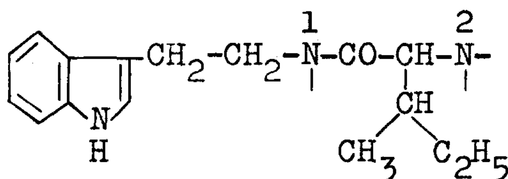
Partial hydrolysis with $Ba(OH)_2$ gave a fraction with positive ninhydrin and Ehrlich reactions. Extraction of this fraction and further hydrolysis

under the same conditions gave isoleucine and tryptamine, and thus these two components are linked by a peptide bond.

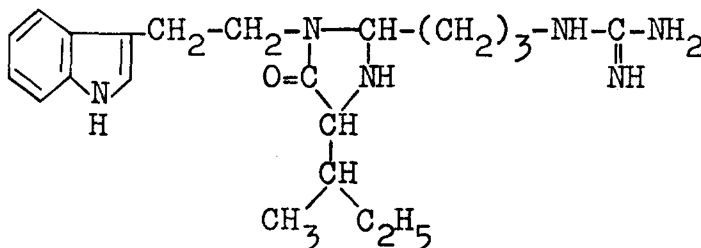
In spite of the positive (but weak) ninhydrin reaction, hydroluciferin contains no primary amino group from the following facts:

(a) After treatment with 2,4-D. N. F. B., hydroluciferin did not afford D. N. P. isoleucine by hydrolysis.

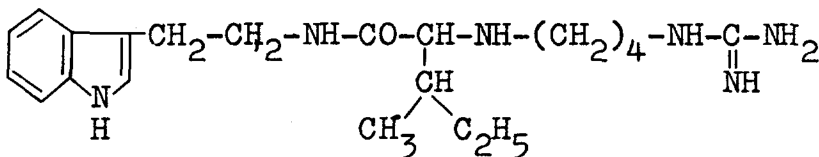
(b) Hydroluciferin did not react with nitrous acid.



Attachment of the γ-guanidino butyric acid group to positions 1 and 2 to afford the cyclic structure is favored by the following facts.



The alternative possibility of attachment to position 2 only would give a chain structure.



which would be rather stable contrary to the extreme instability of hydroluciferin.

Hydroluciferin has pKa's at ca. 5.2 and 12.0 (guanidino group) in 33 % MeOH. The lower pKa' at 5.2 is only explicable by assigning to it the cyclic secondary amino group with a β-NCO group. The pKa's of secondary amines are generally around 9.5. The Dragendorf reaction is positive.

Deduction of the Structure of Luciferin

It appears that the skeletal structure of hydroluciferin is identical with that of luciferin.

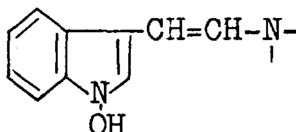
The Adamkiewitz and Ehrlich (Neubauer-Rhode) reaction were negative. This shows that the indole nucleus on luciferin is substituted either at the nitrogen or at the α-position. This substituent should be easily detached by catalytic hydrogenation, since a β-indolethylamine derivative was produced upon hydrogenation of luciferin. This property taken in conjunction with the pKa' at 8.3 in 33 % MeOH is most suitably accounted for by the presence of a N-hydroxy indole group in luciferin. The titration curve shows that the pKa' at 8.3 must be attributed to the acidic group. A few N-hydroxy indole model compounds showed that the pKa' of these are of the order of ca. 8.3, and that the >N-OH group is converted into >NH by catalytic hydrogenation with platinum oxide catalyst. This is further supported by the following facts:

(a) Aromatic primary amine derivatives could not be produced by mild hydrolysis of luciferin with $Ba(OH)_2$; this excludes the possibility of the presence of α-OH group.

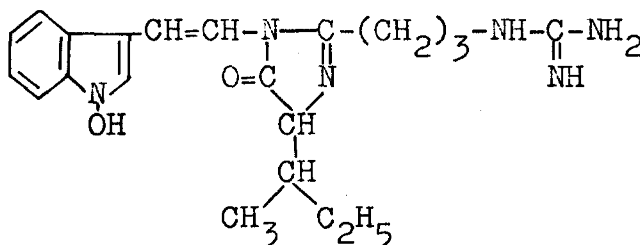
(b) Ferricyanide oxidation of N-hydroxy indole compounds gave

oxidation results similar to those obtained with luciferin.

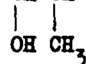
The λ_{max} of luciferin at 435 mμ suggests that the chromophore of the indole moiety is further extended as follows and this also accounts for the production of glycine by hydrolysis and also for the absorption of hydrogen.



Oxidation of luciferin with hydrogen peroxide in the presence of ammonia gave crystalline oxyluciferin C, the hydrolysis of which under various conditions produced no isoleucine. However, the production of isoleucine is resumed if oxyluciferin C is reduced prior to hydrolysis. These facts suggest the presence of an N(isoleucine)-oxide group in oxyluciferin C. The facts that secondary amino groups are not converted into N-oxide under the condition employed and that an additional double bond is present in luciferin (from the molecular formula and hydrogenation of luciferin) lead to the presence of the group N(isoleucine)=C< and thus so the present tentative structure of



The exact position of the double bond in the 4(or 5)-imidazolone ring is not established. It is provisionally placed as shown from the results of hydrolysis which did not afford any acid like HOOC-CH-CH-C_2H_5, and also



in order to account for its rather long wavelength absorption maximum.

The guanidine and oxo-diaza groups are responsible for the formation of a dihydrochloride, whereas the N-OH group in the indole moiety gives rise to the pKa' at 8.3.