Tetrahedron Letters No.29, pp. 3437-3444, 1966. Pergamon Press Ltd.
Printed in Great Britain.

# CYPRIDINA BIOLUMINESCENCE II
# STRUCTURAL STUDIES OF CYPRIDINA LUCIFERIN BY MEANS OF A HIGH RESOLUTION MASS SPECTROMETER AND AN AMINO ACID ANALYZER

Yoshito Kishi, Toshio Goto, Shoji Eguchi*, Yoshimasa Hirata
Chemical Institute, Faculty of Science, Nagoya University
Chikusa, Nagoya, Japan

Eiji Watanabe and Tetsumi Aoyama
Japan Electron Optics Laboratory Co., Ltd.
Akishima, Tokyo, Japan

(Received 5 May 1966)

STRUCTURE of Cypridina luciferin (I) was elucidated as described in the preceding communication (1). During this investigation, analysis of the high-resolution mass spectra of some derivatives of luciferin greatly contributed for determination not only of the molecular formula of luciferin but also elucidation of some parts of the structure. On the other hand, a curious result of the amino acid compositions produced by hydrolysis of luciferin and its derivatives (2) lead some confusions in the early stages of this research, but has now been interpreted using structure Ia for luciferin. In this communication, the interpretation of both of the mass spectra and the results of amino acid analysis will be presented.

Mass Spectrum of Etioluciferamine (IV).

Oxidation of luciferin (I) afforded oxyluciferin (II) and etioluciferin (III). Since the measurement of mass spectrum of III was prevented by the polar nature of the guanidine group in the molecule, III was hydrolyzed by heating with barium hydroxide to eliminate the

*Present address: Department of Synthetic Chemistry, Faculty of Engineering, Nagoya University, Chikusa, Nagoya, Japan

Table 1. Element Map of Etioluciferamine (IV)

| m/e | Intensity | $CHN_5$ | $CHN_4$ | $CHN_3$ | $CHN_2$ | $CHN_1$ |
|---|---|---|---|---|---|---|
| 267 | **** | 15-17 | | | | |
| 250 | ***** | | 15-14 | | | |
| 237 | *** | | 14-13 | | | |
| 224 | **** | | 13-12 | | | |
| 209 | * | | 12- 9 | | | |
| 197 | ** | | | 12-11 | | |
| 196 | * | | | 12-10 | | |
| 183 | *** | | | 11- 9 | | |
| 182 | *** | | | 11- 8 | | |
| 155 | *** | | | | 10-7 | |
| 142 | *** | | | | 9-6 | |
| 141 | ** | | | | | 10-7 |
| 140 | * | | | | | 10-6 |
| 130 | ** | | | | | 9-8 |
| 129 | ** | | | | | 9-7 |
| 128 | * | | | | | 9-6 |
| 127 | ** | | | | | 9-5 |
| 125 | ** | | | 6-11 | | |
| 124 | *** | | | 6-10 | | |
| 118 | * | | | | | 8-8 |
| 117 | ** | | | | | 8-7 |
| 116 | ** | | | | | 8-6 |
| 115 | ** | | | | | 8-5 |
| 114 | ** | | | | | 8-4 |
| 113 | ** | | | | | 8-3 |
| 44 | ***** | | | | | 2-6 |
| 30 | *** | | | | | 1-4 |

Differences between observed and calculated mass values are less than 3 millimass unit.

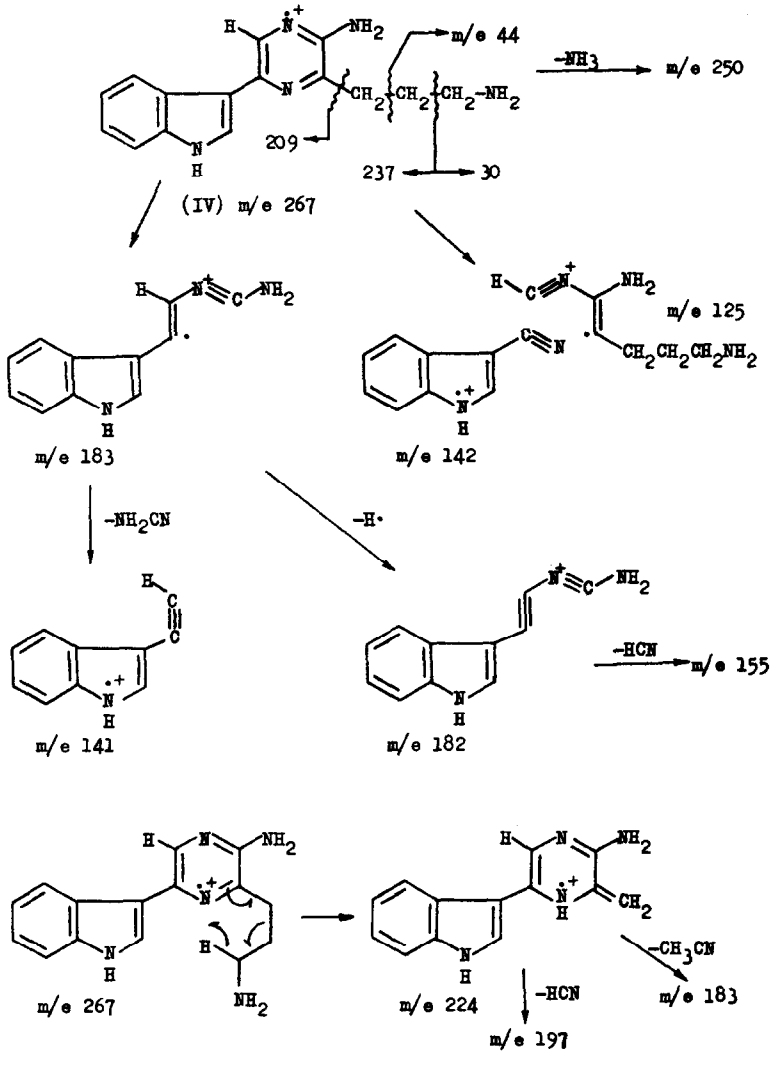

m/e 44
-NH3
m/e 250
209
237
30
(IV) m/e 267
m/e 125
m/e 183
m/e 142
-NH2CN
-H·
-HCN
m/e 155
m/e 141
m/e 182
m/e 267
m/e 224
-CH3CN
m/e 183
-HCN
m/e 197

Chart 1

guanidine group and give etioluciferamine (IV) (= desguanyletioluciferin), (HCl salt, in sealed tube) m.p. 251-252°, which is sufficiently volatile for measurement of its mass spectrum using a Nihondensi JMS-01 mass spectrometer equipped with a direct inlet. In Table 1 is shown the element map of the spectrum of IV, from which the following possibilities are inferred (Chart 1).

The molecular formula of IV is confirmed as $C_{15}H_{17}N_5$. The $C_8H_nN_1$-fragments evidently contain the indole nucleus. Since the largest $N_1$-fragment includes ten carbon atoms, a two-carbon side-chain is extended from the indole nucleus. That nitrogen atoms are attached at the α- and possibly β-carbon atom of the side-chain is deduced from the $N_2$-fragments at m/e 155 and 142. A primary amino group must be present in a terminal position since $C_{15}H_{14}N_4$ ($M^+ - NH_3$) peak is observed. Three carbon atoms are successively eliminated after the loss of the amino group, suggesting the presence of the group $-CH_2CH_2CH_2NH_2$.

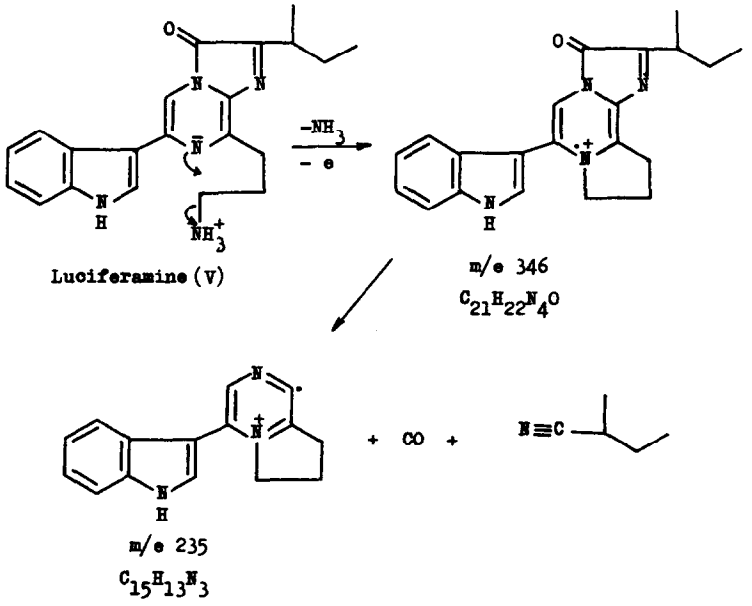
As indicated by Biemann (3), the main fragmentation of an alkylated pyrazine ring is the elimination of RCN molecule. Formation of the ions at m/e 183, 182, 142, and 141 can be explained by this mechanism. Presence of a strong peak at m/e 224 suggests that the McLafferty rearrangement takes place. This type of rearrangement has been observed in the case of pyrazine derivatives bearing an alkyl chain of at least three carbon atoms long (3). Further elimination of HCN or $CH_3CN$ from the m/e 224 ion gives m/e 197 and 183 ion, respectively. The arrangements of the three substituents of the pyrazine ring in IV are thus highly reasonable.

Mass Spectrum of Luciferamine (V).

Hydrolysis of luciferin (I) with barium hydroxide affords luciferamine (V), (HCl salt, in sealed tube) m.p. 238-240° dec., by loss of the guanyl group. Reproducibility of the mass spectra of luciferamine was not very good, but at least two peaks were always observed at m/e 346 ($C_{21}H_{22}N_4O$) and at 235 ($C_{15}H_{13}N_3$). Since luciferamine contains at least five nitrogen atoms (4), the peak at m/e 346 is not the molecular ion, but is considered as the fragment produced by the loss of an ammonia molecule.

Luciferamine (V) has an acidic pKa at 8.0 as well as the basic

pKa at 10.4 corresponding to the amino group produced from the guanidine group by hydrolysis of I (5). The acidic group corresponding to the pKa' 8.0 must be the N-H group in the dihydropyrazine nucleus, which is conjugated with the carbonyl group. Between these two pKas luciferamine exists as a zwitterion as shown in Chart 2. Dissociation of luciferamine hydrochloride in the mass spectrometer may first give the zwitterion, which is then decomposed with elimination of an ammonia molecule to the more volatile, non-ionic compound $C_{21}H_{22}N_4O$ prior electron impact.




Luciferamine (V)

m/e 346
$C_{21}H_{22}N_4O$

m/e 235
$C_{15}H_{13}N_3$

Chart 2

These fragments support the molecular formula of luciferamine, $C_{21}H_{25}N_5O$, and then luciferin, $C_{22}H_{27}N_7O$, deduced in the preceding paper (1).

Acid Hydrolysis of luciferin (I) and Hydroluciferin (VI).

Hydrolysis of luciferin and hydroluciferin with 6N hydrochloric acid at 100° with or without oxygen afforded mixtures of amino acids as shown in Table 2. The complicated results can be interpreted successfully by the structures presented in the preceding paper (1) as shown in the Charts 3 and 4.

Table 2

Amino acids obtained from luciferin and hydroluciferin (2)

| | Luciferin (I)* | | Hydroluciferin (VI)* | |
|---|---|---|---|---|
| | vacuum | $O_2$ | vacuum | $O_2$ |
| Glycine | 0.96mole | 0.93 | 0.03 | 0.12 |
| Arginine | 0.49 | 0.38 | - | - |
| γ-Guanidinobutyric acid | 0.13 | ? | - | 0.10 |
| Proline | - | - | 0.03 | 0.31 |
| Isoleucine | 0.07 | 0.07 | 0.50 | 0.50 |
| Alloisoleucine | 0.09 | 0.09 | 0.48 | 0.46 |
| Ammonia*** | 0.27 | 0.33 | 0.40 | 0.41 |
| | | | ** | ** |

*Amino acids less than 0.03 mole in quantity are omitted from the table.

**Measurement of the quantities of ammonia is not so accurate as that of other amino acids.

***Production of about 0.2 mole of 1-methylhistidine was reported, but the product was not rigidly identified and interpretation of its formation is difficult.

Arginine, γ-guanidinobutyric acid, and proline must be derived from the arginine moiety. Since the α-carbon in the isoleucine moiety is not asymmetric, isoleucine and alloisoleucine are always produced in nearly the same amounts.

## References

1. Y. Kishi, T. Goto, Y. Hirata, O. Shimomura and F. H. Johnson, Tetrahedron Letters
2. S. Eguchi, J. Chem. Soc. Japan 84, 86 (1963).
3. K. Biemann in F. W. McLafferty, "Mass Spectrometry of Organic Ions", Academic Press, New York (1963). p. 534.

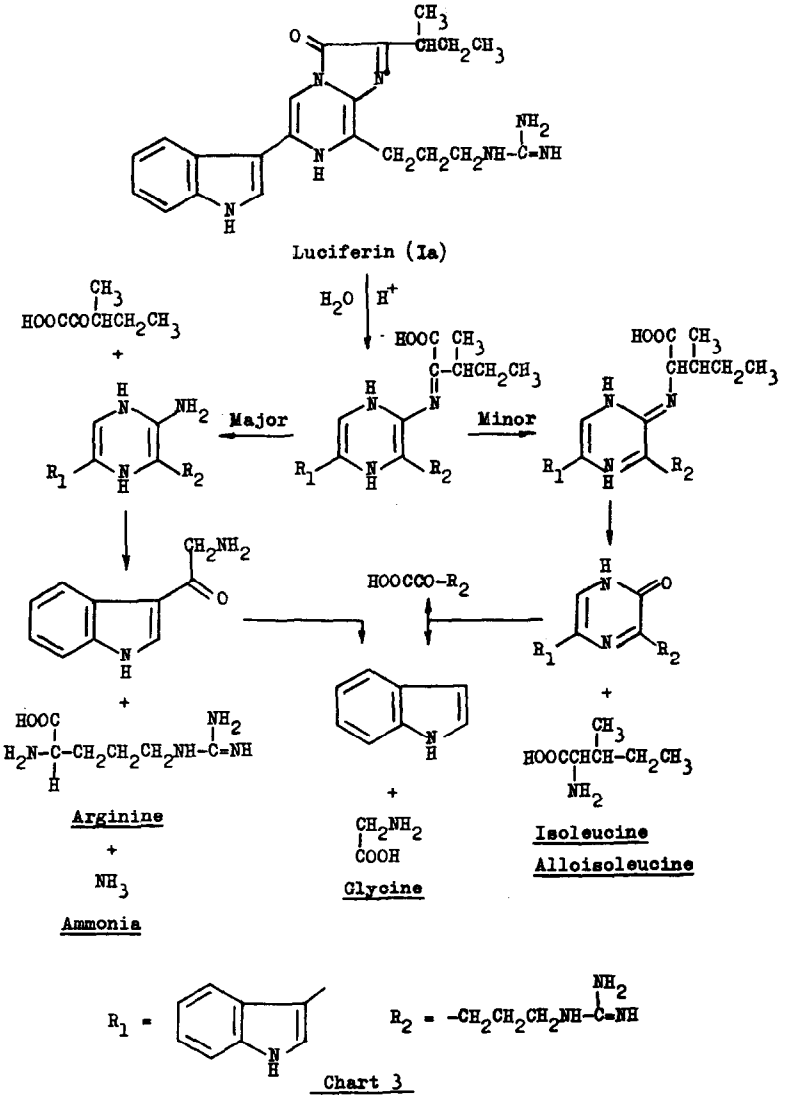



Chart 3

Acid Hydrolysis Products of Luciferin

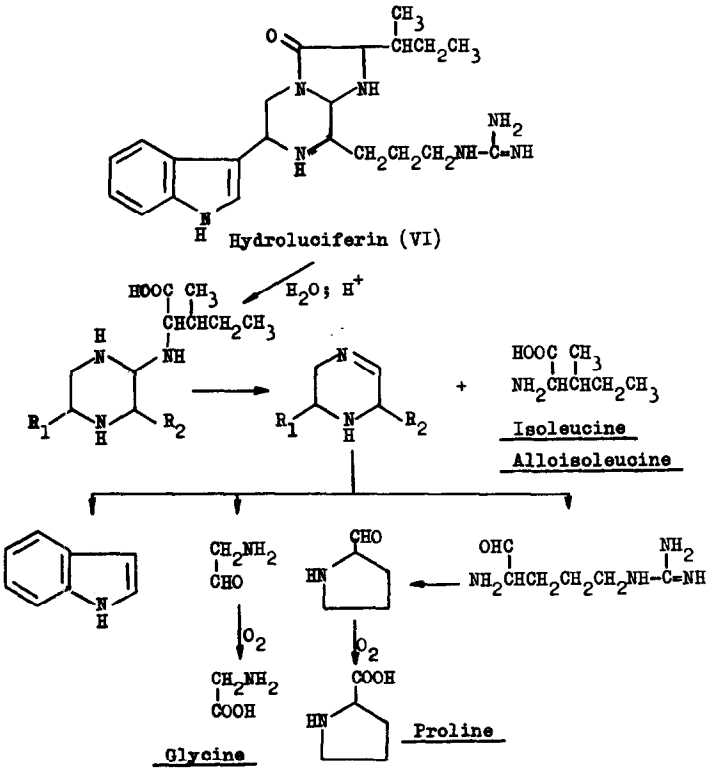



Chart 4

Acid Hydrolysis Products of Hydroluciferin

4. Etioluciferamine (IV), which contains five nitrogen atoms, can be obtained from luciferin without using any nitrogen-containing reagents.
5. pKa's of luciferin (I) in water: below 2, 8.3 (acidic), and above 11 (guanidine).