Tetrahedron. Vol. 31, pp. 939 to 943. Pergamon Press 1975. Printed in Great Britain

CYPRIDINA BIOLUMINESCENCE—IX

TAUTOMERIC STRUCTURES OF 2-METHYL-3,7-DIHYDROIMIDAZO[1,2-a]-PYRAZIN-3-ONE, 2-METHYL-3-AMINOIMIDAZO[1,2-a]PYRAZINE AND THEIR DERIVATIVES IN NEUTRAL AND ACIDIC MEDIA

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(*Received in Japan* 20 *August* 1974; *Received in UK for publication* 31 *October* 1974)

Abstract—Tautomeric structures of the title compounds in neutral and acidic media are discussed. In neutral solutions 3 and 4 exist as structure A and E, respectively. In weakly acidic methanol 3 exists as monocation H, whereas in aqueous solution as monocation G. In both strongly acidic aqueous and methanolic solutions 3 exists as dication K. On the other hand, 4 exists in weakly acidic and strongly acidic media as monocation L and dication M, respectively. The above conclusion can be extended to the 6-phenyl and 6-(3′-indolyl) derivatives of 3 as well as *Cypridina* luciferin (1) in aqueous and methanolic solutions.

Cypridina luciferin, a bioluminescent substance isolated from *Cypridina hilgendorfii*, was deduced to have structure **1**.[1] Further investigations[2] have shown that 3,7-dihydroimidazo[1,2-a]pyrazin-3-one nucleus (**2**) is the essential part of **1** for light emission and that the three substituents on the nucleus contribute to the high quantum yield and rate of bioluminescence.[3] A few tautomeric structures are possible for the dihydroimidazopyrazinone (**2**) in neutral and acidic media. For further investigations on the mechanism of the light emission of luciferin (**1**), it is important to determine the structures in which luciferin exists in solutions. We have first determined the tautomeric structures of model compounds, 2-methyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (**3**) and 2-methyl-3-aminoimidazo[1,2-a]pyrazine (**4**).

Structure of **3** *and* **4** *in neutral media.*[4] Three possible tautomeric structures, **3-A** to **3-C**, can be written for the compound **3**. The UV spectra of **3** in water, methanol, diglyme,† hexane,† and dimethyl sulfoxide† show no significant difference in shape and wavelength of the absorption, indicating that **3** exists in the same tautomeric structure in these neutral media. In the PMR spectrum of **3** in DMSO-d_6 the signal of the 2-Me protons appears as a singlet [actually a very small long-range coupling (0·6 Hz) with H-8 is observed], so that the possibility of structure **3-C** is excluded. Treatment of **3** with dimethyl sulfate and potassium carbonate gave 7-methyl derivative of **3** (**5**), the structure of which was determined by mass spectroscopy.[4] The UV spectra of **5** in methanol and water are nearly superimposable with those of **3**. Thus, possibility of **3-B** is also excluded and **3-A** should, therefore, be the actual tautomeric structure of **3** in neutral media.

In contrast, 2-aminoimidazo[1,2-a]pyrazine exists in the form **4-E** as deduced from the following evidence. The 3-benzyl derivative **6** is obtained by treating **4** with benzaldehyde followed by hydrogenation.[4] The fact that the benzylic protons of **6** are spin-coupled with the adjacent N-H (J = 6·5 Hz) in the PMR spectrum of **6** in DMSO-d_6 leads to the conclusion that **6** exists as the formula **6**. As the UV spectra of **6** in methanol and in DMSO are essentially superimposable with those of **4**, the

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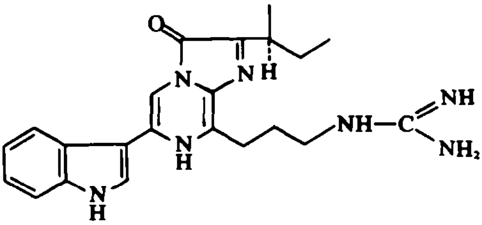
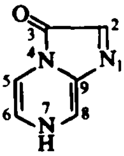
†3 decomposes rapidly in dilute solutions of these solvents.

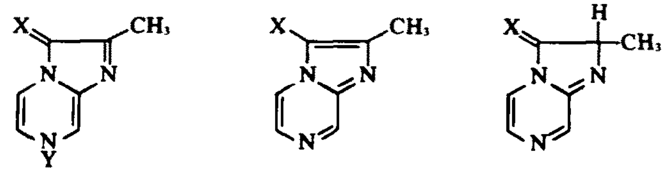


Cypridina luciferin (**1**)



3,7-Dihydroimidazo[1,2-a]pyrazin-3-one (**2**)

Formula 1



3-A: X = O, Y = H
4-D: X = NH, Y = H
5: X = O, Y = CH₃

3-B: X = OH
4-E: X = NH_2
6: X = $NHCH_2Ph$
7: X = Cl

3-C: X = O
4-F: X = NH

FORMULA 2

tautomeric structures of **4** and **6** in these solvents must be in the same form. Hence the tautomeric structure of **4** can be represented by **4-E**.

The prototropic tautomerism of heteroaromatic compounds containing hydroxy and amino groups has been extensively investigated.[5] It has been shown[6] theoretically and experimentally that the amino tautomers are usually more stable than their imino counterparts. On the other hand, in the case of compounds with hydroxy α or γ to nitrogen the lactam or oxodihydro tautomer is favored, but the latter to a much smaller extent. The above results are in good agreement with this generalization.

The relationship between the structure and UV absorption is best interpreted as follows. Each of compounds **3** and **4** has two major resonance structures, A_1 and A_2, and E_1 and E_2, respectively, but the contribution of A_2 and E_1 would be small because of the charge separation on these structures. More contribution of the dihydropyrazinone structure (suffix 1) such as A_1 and E_1 results in more bathochromic shift on the UV spectrum, whereas more contribution of the imidazopyrazine structure (suffix 2) such as A_2 and E_2 causes more hypsochromic shift. Thus, compound **4** absorbs light in much shorter wavelength (346 nm in H_2O) than that absorbed by compound **3** (420 nm in H_2O). The chloro compound **7**, whose structure is fixed to the imidazopyrazine, absorbs at 318 nm (ϵ 2380).

Structure of cations of **3**. Three possible structures can be written for the monocation of **3**, namely, **G**, **H** and **J**, each of which has two mainly contributing resonance structures as indicated by the suffix 1 and 2.

In the case of structure G the contribution of G_2 is much less compared to A_2 in the resonance of A; the absorption maximum should be shifted to a longer wavelength than that of **3** in neutral media. If a proton attaches on the oxygen as in structure **H**, the contribution of resonance structure H_2 should become more important than H_1, since the positive charge on nitrogen should be more stable than that on oxygen; it is expected that the absorption max. is shifted to a shorter wavelength similar to the absorption max. of the neutral 2-amino compound **4**. In the third case, contribution of the resonance structure J_1 may be neglected so that the absorption max. should be the shortest of the three.

In the absorption spectra of **3** in methanol solutions, isosbestic point is observed at 396 nm when acidity is changed between neutral and 0·1N HCl, indicating the equilibrium between the neutral and a monoprotonated species. The monoprotonated species must have the structure **H** since the cation absorbs light in a shorter wavelength (380 nm) than the neutral molecule (428 nm) and since the 7-Me derivative **5** behaves similarly (438 nm in MeOH → 387 nm in acidic MeOH) thus excluding the structure **J**.

In 10N HCl-MeOH, additional proton is attached to the N-1 position to form the dication **K** which shows slightly bathochromic shift (387 nm) than the monocation (380 nm) indicating the more contribution of K_1 than H_1.

In contrast, aqueous solutions of **3** behave differently. The monoprotonated species, which forms in 0·2N HCl solution, has its absorption max. in a longer wavelength (440 nm) than that of the neutral molecule (420 nm). The second protonation, which occurs in 12N HCl, gives the same species (386 nm) to that in 10N HCl-MeOH solution. The 7-Me derivative **5** shows almost identical behavior with **3** in acidic aqueous solutions (430 nm in neutral, 445 nm in 0·2N HCl, and 388 nm in 12N HCl). It is concluded, therefore, that the monocation of **3** in aqueous solution has the structure **G**, which absorbs light in the much longer wavelength than **A** owing to the less contribution of G_2 than A_2. That the structure of the monocation in aqueous solutions differs from that in methanol and that there exists an equilibrium between

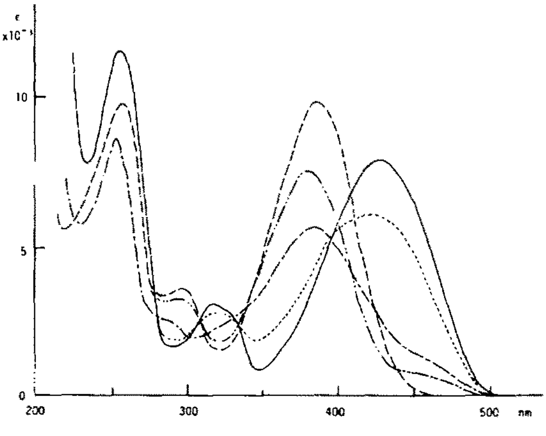
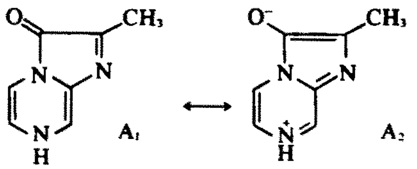
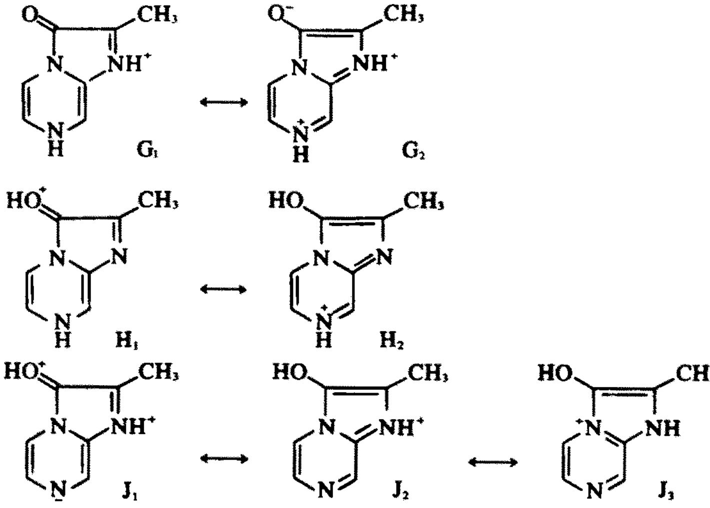


Fig. 1. **3** in methanol. ——— neutral; ---- 0·001N HCl; ——0·01N HCl; ——— 1N HCl; ——— 10N HCl.

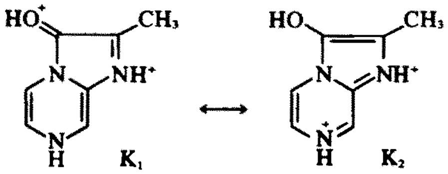
Neutral molecule of 3



Monocation of 3



Dication of 3



FORMULA 3

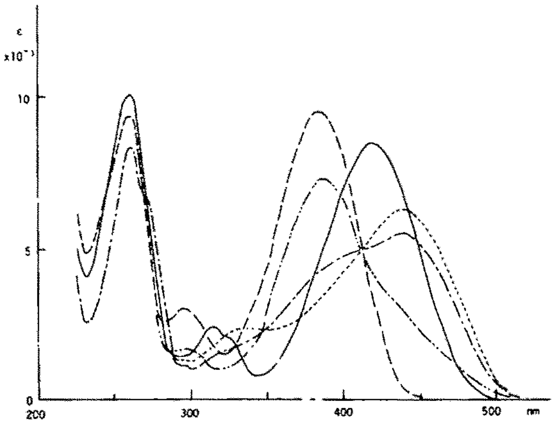


Fig. 2. 3 in water. ——— neutral; ----- 0·2N HCl; ——— 2N HCl; —·—·— 6N HCl; —·—·— 12N HCl.

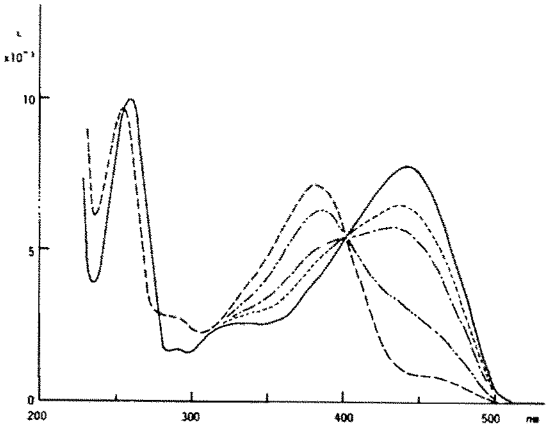


Fig. 3. 3 in aqueous methanol containing 0·2N HCl. ——— 100% water; ----- 75% water; ——— 50% water; ——— 25% water; —·—·— 0% water.

these two cations can be demonstrated by changing the solvent from 100% water to 100% methanol, containing 0·2N HCl. As shown in Fig. 3 an isosbestic point is observed at 404 nm.

Structure of cations of **4**. A large bathochromic shift is observed on the UV spectrum of the amino compound **4** by acidification to 0·01N HCl (345 nm in neutral → 400 nm in acidic solution); an isosbestic point being observed at 363 nm and pK'_a at 4·90. The shift indicates that the first protonation occurs on the N-7 (structure L). The second protonation shifts further the maximum to a longer wavelength (430 nm). It is interpreted by 1,7-diprotonated structure M in which less contribution of the structure M_2 than L_2 is expected. The spectra of **4** in acidic methanol are essentially the same in aqueous acids.

Structure of derivatives of **3** *in acidic media.* The 6-phenyl and 6-(3′-indolyl) derivatives of **3** show essentially identical behavior with **3** in acidic aqueous solutions as well as in acidic methanol solutions as shown in Figs. 5 and 6.

The UV spectrum of *Cypridina* luciferin (**1**) is almost identical with that of the 6-(3′-indolyl) derivative of **3**.*

NMR *spectra.* As discussed below, the NMR spectral data, which are summarized in Table 1, are consistent with the structures deduced from the UV spectral data. Very interestingly, the coupling constants, $J_{6,8}$ and $J_{5,8}$, depend on the structures; in the case of 3,7-dihydroimidazo[1,2-a]pyrazin-3-one structure (**2**) an appreciable spin-coupling (~ 1·5 Hz) is observed between H-6 and H-8, but almost no coupling between H-5 and H-8. On the contrary, the compounds having imidazo[1,2-a]pyrazine structure show almost no coupling between

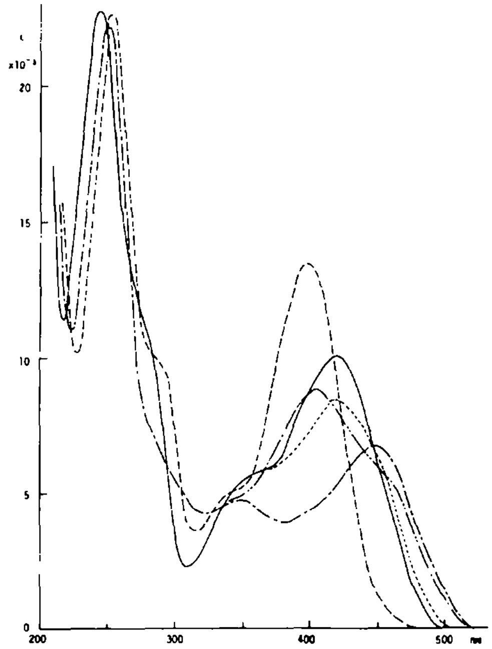


Fig. 5. 6-Phenyl-3 in water. ——— neutral; ---- 0·01N HCl; ——— 1N HCl; ——— 4N HCl; ——— 12N HCl.

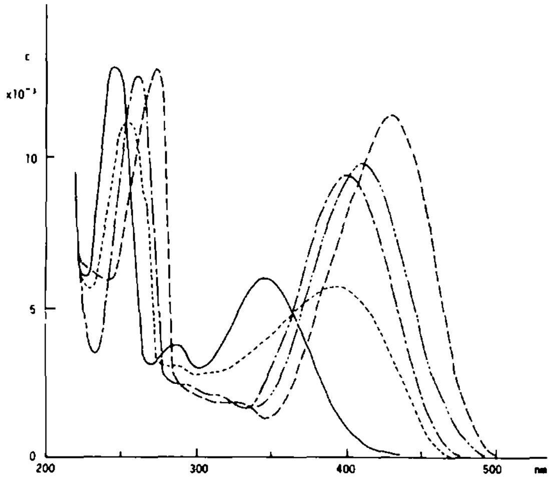
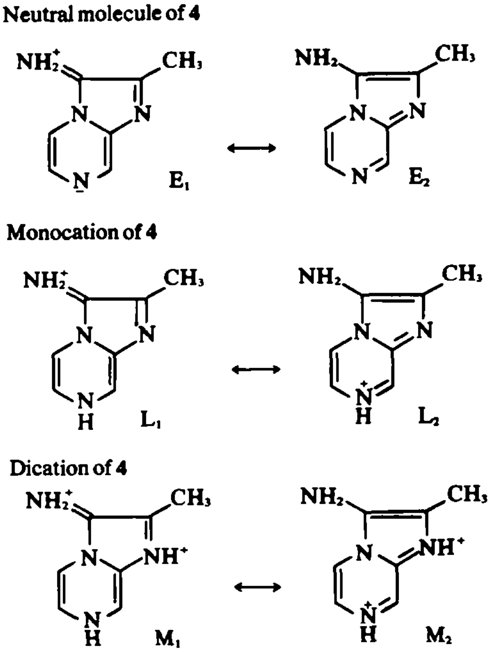


Fig. 4. **4** in water. ——— pH 8; ----- pH 5; ——— 0·01N HCl and 0·1N HCl; ——— 2N HCl; ——— 12N HCl.



FORMULA 4

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*Crystalline *Cypridina* luciferin dihydrochloride has brilliant orange color whereas only very faint brown color is observed on the crystals of the luciferin dihydrobromide. This difference is best interpreted by assigning the structure **H** for the dihydrochloride and structure **J** for the dihydrobromide. When dissolved in solvents the dihydrobromide gives the same orange yellow solutions to that obtained from the dihydrochloride. Preliminary discussions appeared in: Y. Kishi, T. Goto, Y. Hirata, O. Shimomura and F. H. Johnson, *Bioluminescence in Progress* (Edited by F. H. Johnson and Y. Haneda), pp. 89–113. Princeton University Press, N.J. (1966).

Table 1. NMR spectra of imidazo[1,2-a]pyrazine derivatives (60 MHz in DMSO-d_6; δ in ppm from int. TMS; J in Hz)

Compound	Mainly contributing resonance structure	$J_{5,6}$	$J_{6,8}$	$J_{5,8}$	$J_{2\text{-Me},8}$	$\delta_{2\text{-Me}}$	δ_5	δ_6	δ_8
3	1	5·6	1·0	<0·4	0·6	2·31	7·21	6·80	7·73
6-Me-**3**	1	1·0(5,6Me)	—	~0	0·6	2·27	6·98	2·08(Me)	7·62
5	1	6·0	1·5	~0	0·7	2·28	7·22	6·70	7·71
4	2	4·6	~0	1·4	0	2·36	8·05	7·69	8·65
7	2	4·6	~0	1·4	0	2·43	8·38	8·03	9·02
3·HCl	1 < 2	5·4	0·6	0·8	~0	2·5	8·23	7·67	8·87
6-Ind-**3**·HCl	1 < 2	—	—	1·0	~0	2·43	8·42	—	8·85
Des-Me-**4**·HCl	1 ≃ 2	5·6	0·8	0·8	~0	7·87(2-H)	8·67	7·78	9·08

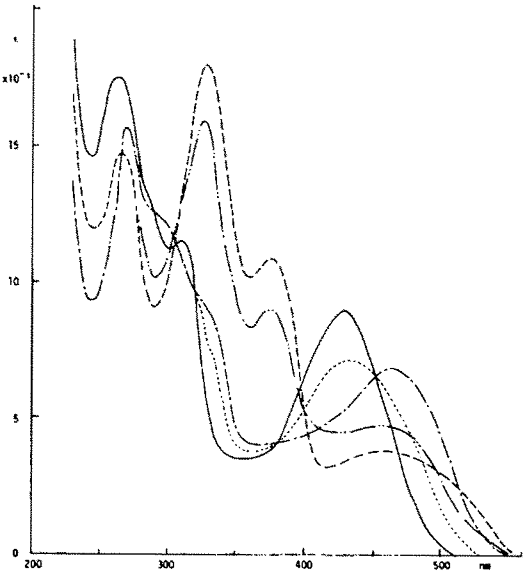

Fig. 6. 6-(3′-Indolyl)-**3** in water. —— neutral; ----- 0·01N HCl; ——0·1N HCl and 1N HCl; ——6N HCl; ——— 12N HCl.

H-6 and H-8, whereas *ca* 1·4 Hz is observed as the coupling between H-5 and H-8.

In the case of 6-membered nitrogen heteroaromatics such as pyridine and pyrimidine, para-coupling ($J_{2,5}$) is *ca.* 1 Hz whereas no meta-coupling ($J_{2,6}$) is observed.[7] This tendency is thus applicable to that of the fully aromatic imidazopyrazine structure. That 6-Me-**3** shows 1·0 Hz coupling between the 6-Me protons and H-5 suggests the double bond character and not an aromatic character of the 6,7-bond in 6-Me-**3** and hence in **3**.

Similarly, in the 3,7-dihydro structure a long-range coupling (*ca.* 0·6 Hz) is observed between the 2-Me protons and H-8, whereas no coupling is observed between the 2-Me protons and H-8 in the imidazopyrazine structure.

The hydrochlorides of **3**, 6-(3′-indolyl)-**3** and desmethyl-**4** show very similar coupling patterns each other showing 0·6–1·0 Hz in both $J_{6,8}$ and $J_{5,8}$, suggesting that the structure of these compounds may be an intermediate between the 3,7-dihydro structure and the fully aromatic imidazopyrazine structure. This is consistent with the proposed structures, **H** and **L**, in which both resonance structures, 1 and 2, are mainly contributing.

The assignment of the protons at 5 and 6 positions in **3** was carried out by comparison of the chemical shifts of these protons in the NMR spectra of **3** and 6-Me-**3**.

EXPERIMENTAL

The UV spectra were recorded on Yanagimoto UV/ORD 185-A and Hitachi EPS-3T spectrophotometers. The NMR spectra were taken with a Varian A-60 spectrometer; the chemical shifts were given in ppm relative to an internal TMS standard and coupling constants were given in Hz.

Synthesis of the compounds, **3**, **4**, **5**, **6**, **7**, 6-Me-**3**, 6-(3′-indolyl)-**3**, and 2-desmethyl-**4** were reported previously.[8]

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