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CYPRIDINA BIOLUMINESCENCE III
TOTAL SYNTHESIS OF CYPRIDINA LUCIFERIN

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CYPRIDINA luciferin (I) has been shown to have the structure represented by Ia (1). We wish to report in this communication a total synthesis of this bioluminescent substance.

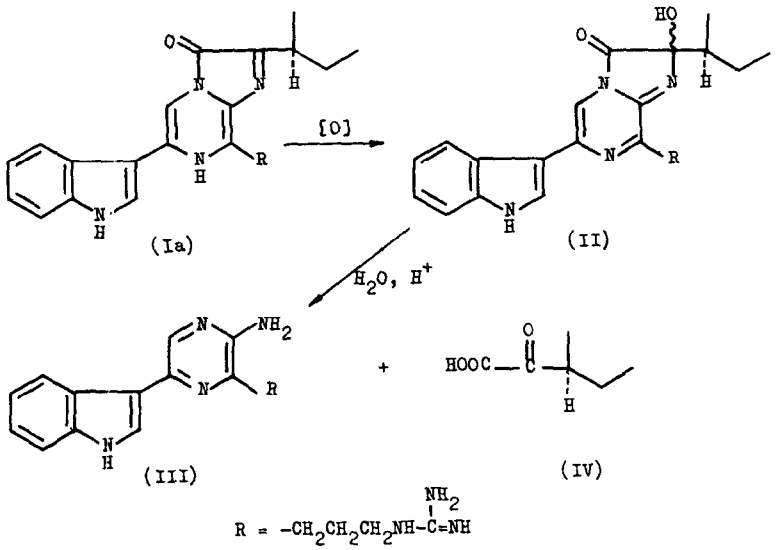
When mixed with Cypridina luciferase in aqueous solution in the presence of oxygen, Cypridina luciferin (I) gives, with emission of light, Cypridina oxyluciferin (II), which could be converted by acid treatment to Cypridina etioluciferin (III) and α-keto-β-methylvaleric acid (IV).

Luciferin (I) is formally constructed from three amino acid conponents (or their equivalents), i. e., tryptamine, arginine and isoleucine moieties. The route of synthesis of luciferin is so designed that the starting materials to be used are those related closely to the three amino acid components. Thus, it consists in two major steps: the synthesis of etioluciferin (III) from those corresponding to tryptamine and arginine, and then condensation of etioluciferin with the keto acid (d-form) (IV) corresponding to isoleucine, to give luciferin.

Synthesis of Cypridina etioluciferin (III).

Carbobenzoxylation of δ-benzoylamino-α-aminovaleronitrile (2), viscous oil, prepared from γ-aminobutyraldehyde diethyl acetal gave α-carbo-

benzoxyamino derivative (V)*, m.p. 108-110°; Mass M^+=351; ν^{KBr} cm^{-1} 3320, 2230, 1715, 1690, 1635; which was converted to the amidine hydrochloride (VII), m.p. 105° (hydrate form*); ν^{KBr} cm^{-1} 3500-2700, 1720, 1685, 1625;



through the imino ether hydrochloride (VI), by the usual way. Selective hydrolysis of the amidine (VII) with hydrogen bromide in acetic acid gave δ-benzoylamino-α-aminovaleramidine dihydrobromide (VIII), m.p. 200-203° (amorphous); ν^{KBr} cm^{-1} 3500-2400, 1690, 1650.

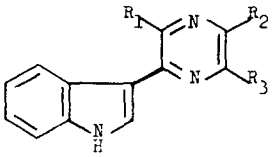
On the other hand, β-indolylglyoxal (IX), m.p. 105-110°(4), was prepared from β-chloroacetylindole (3) by successive treatments with pyridine, p-nitrosodimethylaniline, and dil. sulfuric acid according to Sanna's method (4). Formation of the glyoxal (IX) was recognized by the conversion of the product to a quinoxaline derivative (X)*, yellow needles, m.p. 202-203°; ν^{KBr} cm^{-1} 1508, 1440, 1163, 1130, 755, 740; Mass M^+=245; $\lambda_{max.}^{MeOH}$ mμ (ε) 218 (44,200), 255sh (12,400), 271sh (14,700), 278 (15,700), 310 (10,700), 385 (13,500); by heating with o-phenylene-

* Satisfactory analyses were obtained with the compounds indicated by asterisks.

diamine. As the free glyoxal is considerably unstable, it was converted to its bisulfite addition compound, white powder; $\lambda_{max.}^{H_2O}$ mμ (ε) 248 (11,400), 265 (9,500), 314 (12,200); by treatment with sodium bisulfite solution.

Condensation of the glyoxal-sodium bisulfite adduct with the α-aminoamidine (VIII) in water containing potassium hydroxide (3 eq.) afforded 2-amino-3-(γ-benzoylaminopropyl)-5-(β-indolyl)pyrazine (XI), which was isolated as the picrate,* m.p. 228-229°, and as the nitrate,* double m.p. 167-169° and 234-237°; Mass M^+=371; $\lambda_{max.}^{0.1N\ HCl-MeOH}$ mμ (ε) 223 (33,600), 270sh (14,500), 306 (18,000), 410 (4,100); $\lambda_{max.}^{0.1N\ NaOH-MeOH}$ 226 (31,400), 271 (16,400), 365 (6,300); ν^{KBr} cm^{-1} 3500-3000, 1660-1620, 1540. Hydrolysis of the benzoyl group in the pyrazine (XI) by refluxing with methanolic potassium hydroxide gave 2-amino-3-(γ-aminopropyl)-5-(β-indolyl)pyrazine (XII'), which was converted by treatment with S-methylisothiourea to 2-amino-3-(γ-guanidinopropyl)-5-(β-indolyl)pyrazine (III'). XII' and III' were completely identical with etioluciferamine (XII) and etioluciferin (III) (m.p., mixed m.p., IR, UV, and p.p.c.).

Among four possible structures, IIIa to IIId, for etioluciferin, IIIc and IIId were rigorously excluded in the preceding paper (1), but slight unreliability has remained on the exclusion of the structure IIIb. Now, IIIb is excluded with certainty by this total synthesis of III, since the possible structures for the synthetic pyrazine are limited to IIIa and IIIc. Interestingly, the isomer corresponding to IIIc was not isolated during this synthesis.

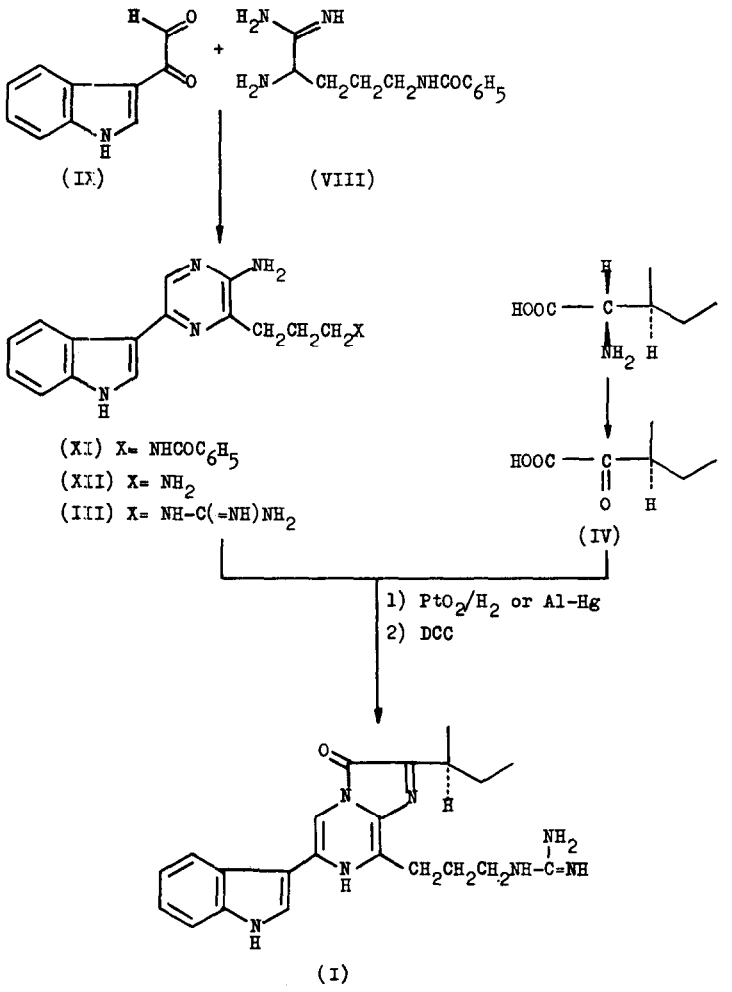


	R_1	R_2	R_3
IIIa	H	NH_2	X
IIIb	X	NH_2	H
IIIc	H	X	NH_2
IIId	X	H	NH_2

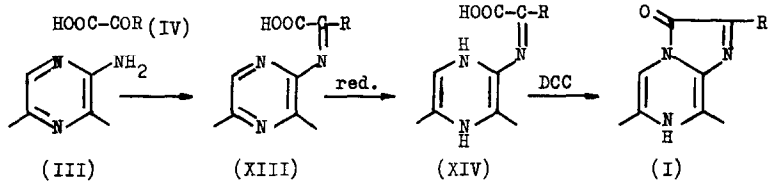
X = $-CH_2CH_2CH_2NHC(=NH)NH_2$

Synthesis of Cypridina luciferin (I).

d-α-Keto-β-methylvaleric acid, α_D +18° (2% solution in 1N HCl) [lit. (5) +20°], was prepared from L-isoleucine by treatment with glyoxylic acid according to Snell's method (6). The keto acid (IV) and


(IX)
(VIII)
(XI) X= NHCOC6H5
(XII) X= NH2
(III) X= NH-C(=NH)NH2
(IV)
1) PtO2/H2 or Al-Hg
2) DCC
(I)

etioluciferin (III) were the starting materials for a sequence of operations leading to the formation of luciferin (I), without isolation of pure intermediates. Thus, addition of etioluciferin (III) into an aqueous solution of the keto acid (IV) yielded the Schiff base (XIII); then careful reduction of the latter by platinum oxide and hydrogen or aluminum amalgam afforded the dihydropyrazine (XIV). After being decanted from the reagent, the solution containing XIV was immediately



treated with dicyclohexylcarbodiimide at 5° for 24 hours to give luciferin (I). The overall yield of luciferin from etioluciferin is less than 1%. The synthetic luciferin was proved to be identical with natural luciferin by means of p.p.c.[n-BuOH-AcOH-H_2O (4:1:2) Rf=0.78, EtOAc-EtOH-H_2O (5:2:3) Rf=0.52], UV spectra, and measurements of luminescent rates (Fig. 1).

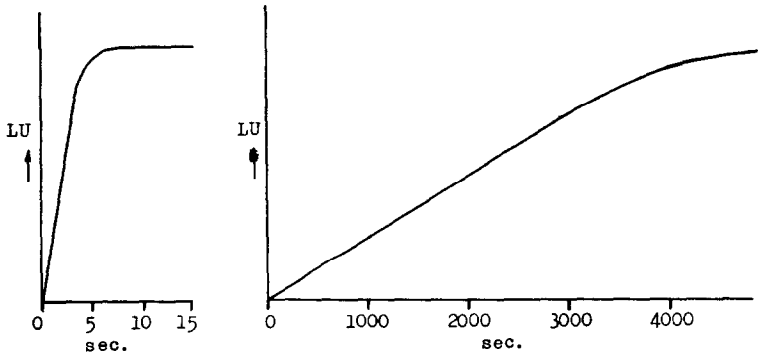


Fig. 1. Relative luminescent rates
Left Fig.: Luciferin (natural and synthetic)
Right Fig.: Luciferamine (natural)
0.1M Phosphate buffer (pH 7.0) containing 0.15M NaCl and Cypridina luciferase (ca. 10γ/ml).

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