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Biosynthesis of Luciferin in *Pyrophorus Pellucens*

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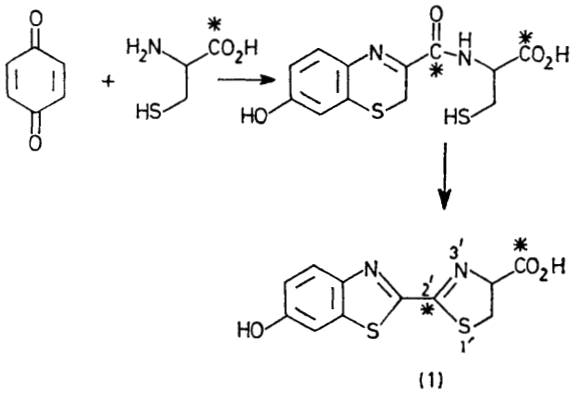
Summary The luminescent click beetle, *P. pellucens*, incorporates DL-cystine into the substrate in the light reaction, showing that luciferin is synthesised in adult beetles.

The mechanism of light emission during the oxidation of firefly luciferin has been reasonably well established,[1] but much less is known about the biosynthetic origin of this unique molecule. It would also be interesting to know whether the adult insects emerge with sufficient luciferin for use during their lifetime as Seliger has estimated[2] or whether luciferin is synthesised by the adult beetles as



(1)

Scheme 1.

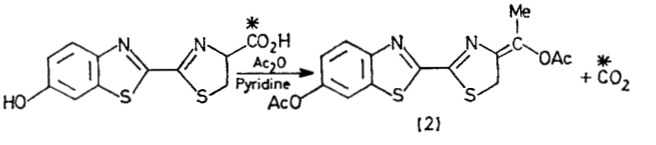
indicated by the work of Okada and his co-workers.[3] We now report a preliminary examination using the large click-beetle, *Pyrophorus pellucens*, from Trinidad.[4] This animal has two yellow-green photophores on the posterior portion of the pro-thorax and an orange-yellow organ on the ventral surface of the thorax. The luciferin from the former organs is identical to that from the other members of the Coleoptera so far investigated.[5]

Table

Compound	Radioactivity[c]/mg
D-Luciferin (**1**)	4305[a]
D-Luciferin	4999[b]
(**2**)	288

[a] 33 beetles; [b] 115 beetles; [c] disintegrations/1000 s after subtraction of background radiation (1010 ± 8d/s).

By the administration of radioactive 2-cyano-6-hydroxybenzothiazole to the Japanese firefly (Luciola species), it has been shown[3] that some synthesis of luciferin may occur. However this compound readily formed luciferin with added cysteine in the buffered solution used in the experiments. The amount of radioactivity obtained *in vitro* was comparable to that of the *in vivo* experiment. Thus confirmation of the synthesis in living animals would be desirable.



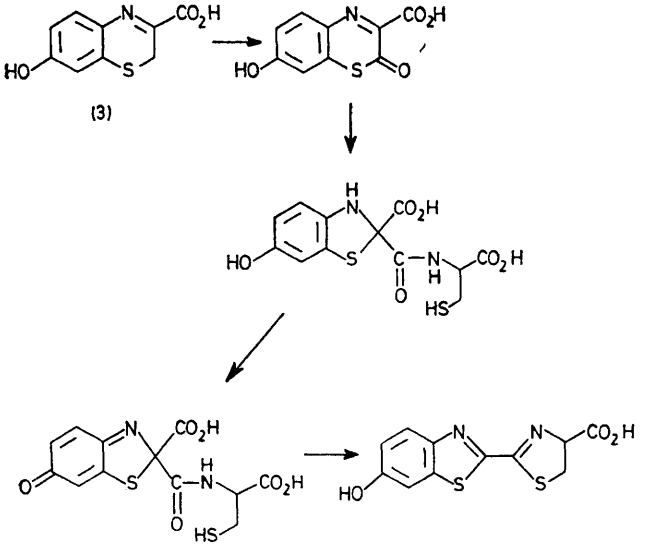
(2)

4652 d/1000s (average) 288 d/1000s

1-[^{14}C]-DL-Cystine hydrochloride (0·05 mC, 1·2 mg) was dissolved in a 10% sucrose solution (10 ml), and 0·1 ml of this solution applied to each of 33 squares of filter paper (3MM, 1 cm^2). The beetles (33) in separate plastic containers were each given a filter paper and were seen to imbibe the solution avidly. Two more applications of the labelled solution were made to the filter paper in 48 h and

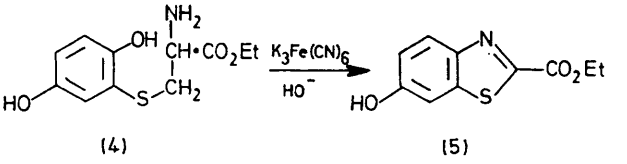
the beetles then dried directly in a desiccator. The photophores were excised, extracted with ethanol and 5 mg of pure D-luciferin added to the residue after evaporation of the solvent. After purification by t.l.c. and crystallisation, it was clear that the luciferin was active, (Table) but owing to the uncertainty of the amount of cystine consumed, it was not possible to give a radioactive yield. Essentially the same experiment was repeated using 115 beetles, with 10 mg of luciferin for dilution.

We can thus confirm that some synthesis of luciferin does occur in this species and perhaps in adult luminescent Coleoptera generally. A preliminary attempt was also made to substantiate our proposal[6] for the biosynthesis of the benzothiazole grouping. This proposal is shown in Scheme 1, and although the order in which the reactions occur is not necessarily specified, the labelling pattern shown should result. We have fully characterised the derivative (**2**) first



SCHEME 2.

briefly reported by the Japanese workers,[3] and the results of the degradation to (**2**) are indicated in the Table. The carbon dioxide was not collected. Two explanations for the observed result seem possible. Either the proposal is essentially correct, and the low value obtained is caused by incorporation of an already present benzothiazole derivative,[3] or the luciferin is more generally labelled to a small extent, and there is no specific labelling at C(2′). We degraded the luciferin to 6-hydroxybenzothiazole in an attempt to answer this question, but insufficient material prevented us recrystallising it to constant activity. Further work may show that C(2′) is not derived from the carboxyl group of cysteine, and we therefore intend to investigate a modified hypothesis (Scheme 2), which shows a feasible route using an alternative carbon atom of the proposed benzothiazine (**3**).



Similar reactions, particularly those of the adducts of aminothiols and *o*-quinones related to dopa have been extensively investigated by Prota and his collaborators[7] during their work on the trichochromes.[8] Although the biosynthesis of these compounds has been examined,[9] luciferin biosynthesis must differ significantly. An earlier attempt[6] to demonstrate a feasible biosynthetic path mistakenly used a benzothiazine dimer[10] as the substrate. We now report that cyclisation and ring contraction can occur in one synthetic operation apparently without involving the dimer. The adduct of cysteine ethyl ester and benzoquinone (**4**) (850 mg characterised and analysed as the trifluoroacetamide) was dissolved in ethanol (20 ml) and aqueous $K_3Fe(CN)_6$ (18 ml, 1 M), made basic with 4 N NaOH (1·2 ml), added. Work up after 1 h gave 67% of the 6-hydroxybenzothiazolyl ester (**5**) in crude form. The analytically pure material (20% yield) gave the expected analysis and showed satisfactory i.r., u.v., n.m.r., and mass spectra. Treatment of the benzothiazine dimer under identical conditions gave very little change in 5 h, as evidenced by the almost unchanged u.v. spectrum. Traces of the benzothiazole were observed by t.l.c. but on this basis the dimer is not an intermediate under these conditions.

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[4] We thank Dr. Fred Bennett, Entomologist in Charge, Commonwealth Institute for Biological Control, Curepe, Trinidad for arranging collections of the insects.
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