Biosynthesis of Firefly Luciferin. Probable Formation of Benzothiazole from *p*-Benzoquinone and Cysteine

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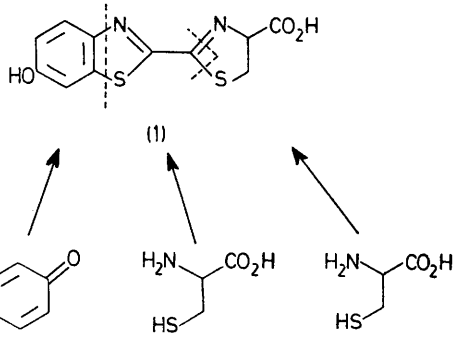
Summary Experiments showing positive incorporation of [^{14}C]-*p*-benzoquinone into luciferin strongly suggest that the benzothiazole nucleus in firefly luciferin arises from *p*-benozquinone and cysteine.

IN considering the biosynthetic route to firefly luciferin (1),[1] it is reasonable to assume that the thiazoline ring is derived from cysteine.[2] However, experiments showing the direct precursor to the benzothiazole ring have not been reported, except McCapra's successful biomimetic synthesis[3] of 2-ethoxycarbonyl-6-hydroxybenzothiazole from benzoquinone and cysteine, both of which were thought to be precursors for the 6-hydroxybenzothiazole unit in firefly luciferin. We now present evidence in support of the above proposition obtained from feeding experiments *in vivo*.

^{14}C-Labelled substrate was injected into live Japanese fireflies, *Luciola cruciata*. After adequate intervals, a number of fireflies were quickly frozen in solid CO_2 and then luciferin was extracted in the usual way.[2] The crude luciferin was diluted with a known amount of cold luciferin and the mixture was transformed into the stable luciferin acetate[2] which was recrystallised from methanol to constant radioactivity. The results are summarised in the Table.

Experiments 1 and 2 (Table) show that benzoquinone and hydroquinone (equivalent to benzoquinone *in vivo*) can be efficiently used for generation of luciferin, and



experiment 3 shows that tyrosine is less effective than benzoquinone. This indicates that benzoquinone is the precursor of luciferin.[4] It is also evident that incorporation of benzoquinone is not through acetate, because the radiochemical yield of luciferin from sodium [^{14}C]acetate is very low (Table, experiment 4).

Thus, the proposition that the benzothiazole ring is formed as a result of the condensation of *p*-benzoquinone with cysteine seems most probable, but confirmation of the exact pathway must await alternative feeding experiments using ^{13}C labelled substrates since it is difficult to obtain degradation products from luciferin.[1]

(*Received, 19th August* 1975; *Com.* 957.)

TABLE. Incorporation of ^{14}C substrate into firefly luciferin.

Experiment no.	Substrate	Activity administered (d.p.m.)	Number of fireflies used	Incorporation of ^{14}C (%) After 24 h	After 60 h
1	[2,3,5,6-^{14}C]-*p*-Benzoquinone	$0{\cdot}95 \times 10^6$	50	0·305	[a]
2	[2,3,5,6-^{14}C]Hydroquinone	$2{\cdot}87 \times 10^6$	50	0·073	0·418
3	L-[*U*-^{14}C]Tyrosine	$2{\cdot}2 \times 10^6$	50	0·005	0·020
4	Sodium[2-^{14}C]acetate	$2{\cdot}2 \times 10^6$	50	0·0023	0·0036

[a] Injecting *p*-benzoquinone in 40% ethanol resulted in the death of many fireflies and the activity could not be measured.

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