Oplophorus Luciferin, Bioluminescent Substance of the Decapod Shrimps, *Oplophorus spinosus* and *Heterocarpus laevigatus*

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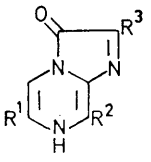
Summary *Oplophorus* luciferin is shown to be 8-benzyl-2-(*p*-hydroxybenzyl)-6-(*p*-hydroxyphenyl)imidazo[1,2-*a*]-pyrazin-3(7*H*)-one.

MANY species of bioluminescent shrimps are known among the euphausids and decapods. Two species of deep-sea decapods, *Systellaspis debilis* and *Oplophorus gracilorostris*, have been shown to give a luciferin–luciferase (L–L) reaction to produce *in vitro* bioluminescence.[1] Johnson *et al.*[1] extracted crude luciferin from the latter shrimp. Later, Yamaguchi proposed structure (I) for this luciferin without conclusive evidence.[2] We have isolated pure luciferin from each of the title shrimps and identified it as compound (II). This is the first report that proves (II) to be a true luciferin, the substrate for an L–L reaction, although there have been several reports concerning compound (II). Thus, (II) was assumed to exist in a modified form in the *Aequorea* photoprotein, aequorin;[3] it was isolated from liver of the squid,

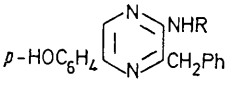
Watasenia scintillans,[4] and considered as a possible precursor of *Watasenia* luciferin (III);[5] *Renilla* luciferin, which was isolated as its sulphate from *Renilla reniformis*, could have the same structure as (II),[3] although Hori *et al.* assigned structure (IV) to it;[6] and *Cavernularia* luciferin was also assumed to be (II).[7] Interestingly, the luciferin from the crustacean decapods is closely related to that of coelenterates rather than that of crustacean *Cypridina*.



(I) $R^1 = R^2 = H$, $R^3 = Pr^nCH(OH)$
(II) $R^1 = p\text{-}HOC_6H_4$, $R^2 = PhCH_2$, $R_3 = p\text{-}HOC_6H_4CH_2$
(III) $R^1 = p\text{-}HO_3SOC_6H_4$, $R^2 = PhCH_2$, $R^3 = p\text{-}HO_3SOC_6H_4CH_2$
(IV) $R^1 = p\text{-}HOC_6H_4$, $R^2 = R^3 = PhCH_2$
(V) R^1 = indol-3-yl, $R^2 = H_2NC(:NH)NH[CH_2]_3$, $R^3 = Bu^s$

The title shrimps gave an L–L reaction (λ_{max} 465 nm) and a cross L–L reaction. Furthermore, cold-water extracts that contain luciferase were found to give luminescence with (synthetic) *Watasenia* preluciferin (II)[4] (λ_{max} 465 nm), whereas no light was observed from the extracts by addition of *Cypridina* luciferin (V) or *Watasenia* luciferin (III), suggesting that *Oplophorus* luciferin was identical with or similar to (II). Lyophylized viscera of these shrimps were washed with CH_2Cl_2 and extracted with methanol. Silica gel column chromatography and three successive t.l.c. separations of the extracts using oxygen-free MeOH–CH_2Cl_2 (1 : 10), acetone–CH_2Cl_2 (1 : 10), and MeOH–benzene (1 : 5) as solvents (R_f values: 0·37, 0·09, and 0·29, respectively) gave a yellow solid, which gives luminescence with cold extracts of the shrimps. Yields were *ca.* 25 μg from 9 individuals of *H. laevigatus* and *ca.* 15 μg from 60 individuals of *O. spinosus*.

The structure of this compound was identified as (II) from the following observations: (a) R_f values on t.l.c. (*vide supra*) were identical with synthetic (II); (b) mass spectra gave an M^+ ion at *m/e* 423; (c) u.v. spectra were superimposable with the published spectra[4] of (II); (d) luminescence rate and relative quantum yield in the L–L reaction were the same as those for (II); and (e) it was oxidized in part on t.l.c. plates to give two fluorescent compounds, which were identified as *Oplophorus* oxyluciferin (= coelenteramide)[8] (VI) and etioluciferin (= coelenteramine)[9] (VII) by comparison of R_f values on t.l.c. and mass spectra (M^+: *m/e* 411 and 277, respectively) with synthetic (VI)[8] and (VII).[9] Structure (I) proposed by Yamaguchi[2] is obviously erroneous.



(VI) R = $p\text{-}HOC_6H_4CH_2CO$
(VII) R = H

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