Research article

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Received: 23 September 2013, Revised: 13 February 2014, Accepted: 20 February 2014 Published online in Wiley Online Library 24 April 2014

(wileyonlinelibrary.com) DOI 10.1002/bio.2671

Characterization of an anthraquinone fluor from the bioluminescent, pelagic polychaete *Tomopteris*

Warren R. Francis,[a,b] Meghan L. Powers[a,b] and Steven H. D. Haddock[a]*

ABSTRACT: *Tomopteris* is a cosmopolitan genus of polychaetes. Many species produce yellow luminescence in the parapodia when stimulated. Yellow bioluminescence is rare in the ocean, and the components of this luminescent reaction have not been identified. Only a brief description, half a century ago, noted fluorescence in the parapodia with a remarkably similar spectrum to the bioluminescence, which suggested that it may be the luciferin or terminal light-emitter. Here, we report the isolation of the fluorescent yellow–orange pigment found in the luminous exudate and in the body of the animals. Liquid chromatography-mass spectrometry revealed the mass to be 270 *m/z* with a molecular formula of $C_{15}H_{10}O_5$, which ultimately was shown to be aloe-emodin, an anthraquinone previously found in plants. We speculate that aloe-emodin could be a factor for resonant-energy transfer or the oxyluciferin for *Tomopteris* bioluminescence. © 2014 The Authors. *Luminescence* published by John Wiley & Sons Ltd.

Additional supporting information may be found in the online version of this article at the publisher's web site.

Keywords: Tomopteris; polychaete; bioluminescence; fluorescence; anthraquinone

Introduction

The ocean is rife with luminous animals, most of which emit blue light (1,2). An exception is the annelid worms in the genus *Tomopteris*, a group of pelagic polychaetes of which several species are reported to produce yellow bioluminescence (3). When agitated, these animals can release glowing material into the water that persists for several seconds. The yellow luminescence of *Tomopteris* has been known for some time (4,5), yet is unstudied when compared with bacterial, beetle or coelenterate systems. It was reported by Harvey that homogenates from the polychaete did not show a luciferin–luciferase-type reaction nor did they produce light with the ostracod luciferin (*Cypridina luciferin*), suggesting the possibility that a previously uncharacterized luciferin is used (3). Shimomura (6) also performed some preliminary investigations into the yellow bioluminescence.

The connection between oxyluciferin fluorescence and the bioluminescence has been described for several systems including *coelenterates*, the firefly and luminous bacteria (1). For *coelenterates*, notably *Aequorea*, the bioluminescence spectrum was identical to the fluorescence spectrum of the photoprotein following the bioluminescence reaction, that is, coelenteramide bound by the photoprotein (7). In the case of the firefly, the bioluminescence matches the fluorescence of the oxyluciferin, the oxidized product of the consumable substrate (8,9). Similarly, in bacterial systems, the bioluminescence spectra also matches the fluorescence of a flavin cation, which is oxidized in the reaction and later regenerated (10–12).

With this in mind, Terio examined two fluorophores in *Tomopteris nationalis* specimens, one appearing yellow–green with ultraviolet excitation, the other yellow–orange (13,14). His detailed observation under the microscope revealed that the yellow–orange fluorescent material was located near the photocytes (light-emitting cells), indicating a likely involvement in the bioluminescence. The material had a fluorescence emission maximum between 550 and 570 nm, and appeared similar to the bioluminescence emission. The fluorescence was unchanged in non-polar solvents suggesting the compound was non-polar. Finally, Terio had speculated that this compound might be involved in the luminous reaction, possibly as the luciferin, but it was never characterized further. Although few luciferins have been isolated, it is thought that bioluminescence evolved many times and novel chemistries may still be found (15). Fewer than 10 luciferins have been identified and the discovery and characterization of a novel luciferin would be a substantial advancement in the study of bioluminescence (2).

Here, we report the isolation and characterization of the fluorescent yellow–orange material from whole Tomopterid specimens. We were able to obtain an accurate mass of the compound as well as the molecular formula. Through a comparison of literature spectra and by liquid chromatograph-mass spectrometry (LCMS), we identified the compound as aloe-emodin, a polyhydroxyl-substituted anthraquinone. Finally,

* Correspondence to: S.H.D. Haddock, Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, CA 95039, USA. E-mail: haddock@mbari.org

[a] Monterey Bay Aquarium Research Institute, Moss Landing, CA, 95039, USA

[b] Department of Ocean Sciences, University of California Santa Cruz, Santa Cruz, CA, USA

we speculate on possible roles based on known redox properties and chemiluminescence from other anthraquinones.

Experimental

Samples

Tomopteris specimens were collected in Monterey Bay using remotely-operated vehicles (ROVs) from 1999 to 2011. Many had been caught previously and frozen in liquid nitrogen. The specimens were found between depths of 269 and 1316 m, typically around 400 m. Specimens varied considerably in size, from 3 cm (~ 0.5 g, wet) to > 40 cm (~ 50g, wet). Polychaete taxonomists recognize that there are several undescribed species in these waters (EV Thuesen and KJ Osborn, pers. comm.) and all tested species had the same luminescent properties, so no attempt was made to discern species. The condition and amount of extractable material were also variable, due to specimens often releasing luminous material prior to being caught or being damaged by the sampling apparatus.

Chemicals

Water for high-performance liquid chromatography (HPLC) was purified by reverse-osmosis. All other solvents were HPLC grade and were purchased from Fisher Scientific. Aloe-emodin was purchased from Sigma-Aldrich.

Extractions

Luminous material was collected when released from live animals in a tube with gentle agitation. Frozen specimens were homogenized using a tissue grinder. The homogenate was divided into microfuge tubes and an equal volume of methyl acetate (MeOAc) was added to each tube, typically 1 mL. This formed emulsions. The tubes were briefly vortexed and then centrifuged for 2 minutes at 16,000***g***. This separated the emulsion into three layers: aqueous, lipids and debris, and organic. The MeOAc layers (organic) were pooled and dried under vacuum at ambient temperature. The sample was reconstituted with three extractions of 20 μL MeOH and transferred to a HPLC vial for injection.

Purification

HPLC was performed using a Shimadzu Nexera system with a Hypersil Gold C_{18} column (50 × 2.1 mm, 1.9 μm Thermo). Run parameters were: 1 mL/min flow rate; binary gradient of H_2O: MeOH + 0.1% formic acid from 95:5 to 5:95 over 10 min; 60°C column temperature; 450 nm fluorescence excitation; 548 nm fluorescence detection; photodiode array scans from 210 to 800 nm at 250 scans/min.

Spectra

The fluorescence and in vivo bioluminescence spectra were acquired using a Ocean Optics QE65000 spectrometer with attached fiber optic. The associated Ocean Optics program SpectraSuite was used to collect spectra. The absorption spectra were measured in a 1mL cuvette on a Tecan Infinite 200 running Tecan i-control software. The digitized data from Terio (13,14) were captured with ImageJ using the "Measure" and "Plot Profile" commands to generate a graph of the intensity across the photograph from the original papers.

Mass analysis

Low-resolution mass was determined by LCMS using a Thermo Finnigan LC/MS/MS (LTQ) electrospray ionization (ESI) mass spectrometer (Thermo, San Jose, CA, USA). For the LC, a Hypersil Gold C_{18} column (50 × 2.1 mm, 1.9 μm, Thermo) was used, and run parameters were: 0.5 mL/min flow rate; binary gradient of H_2O: MeOH + 0.1% formic acid from 95:5 to 5:95 over 28 min; 60°C column temperature. For the MS: negative ionization mode (M-H); source voltage 5.0 kV; mass range from 150.0 to 1000.0 *m/z*; photodiode array range from 200 to 600 nm; normalized collision energy of 35% for MS/MS.

For accurate mass determination, the sample was dried under vacuum and sent out to the Vincent Coates Foundation Mass Spectrometry Laboratory at Stanford University (http://mass-spec.stanford.edu). The sample was reconstituted in 100 μL of 1: 1 H_2O: MeOH and sonicated for 10 min immediately prior to analysis.

For mass profile determination, another dried sample of the HPLC purified compound and a standard of aloe-emodin were sent for LCMS to the Vincent Coates Foundation Mass Spectrometry Laboratory at Stanford University. The sample and standard were reconstituted in 50 μL 1:1 H_2O: MeOH, vortexed for 30 s then sonicated for 10 min. A portion was diluted 1:10 with H_2O:MeOH and transferred into an HPLC vial. For the LC, a Agilent C_{18} column (50 × 2.1 mm, 1.8 μm) was used, with parameters: 0.2 mL/min flow rate; binary gradient of H_2O: acetonitrile + 0.1% formic acid from 90:10 to 0:100 over 10 min. The mass was analyzed with a Bruker MicroTOF-QII quadrupole time of flight mass spectrometer in negative ESI mode.

Results

Acquisition of raw material

Specimens were caught at depth using ROVs. This often enabled careful capture of very large specimens that could be returned to the lab in excellent condition. When agitated, luminescence begins in the parapodia and nearly all of our specimens released glowing material from their parapodia (Fig. 1B). To our knowledge, there is no mention in the literature of these animals releasing luminescent particles. We consider that this may due to the majority, possibly all, of the specimens in the literature being agitated or injured during capture using plankton nets.

We acquired the bioluminescence spectra of the luminous exudate (shown in Fig. 2A, λ_{max}: 565 nm), which is in good agreement with the Atlantic species *Tomopteris nisseni* measured by Latz (16). The bioluminescence spectrum also matches perfectly with the digitized fluorescence spectrum of the yellow–orange fluor measured by Terio (14), to the extent that the image may be converted to a spectrum.

Because live specimens release glowing material, we reasoned that the light emitter could be isolated from the exudate. Luminous exudate has a bright yellow–orange fluorescence under blue light, however, the quantity obtained was insufficient for further analysis. Whole animals displayed a bright yellow–green fluorescence around the coelom in the parapodia even when fixed or frozen (Fig. S1A). This material was clearly visible as a bright yellow pigment in the parapodia for frozen

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

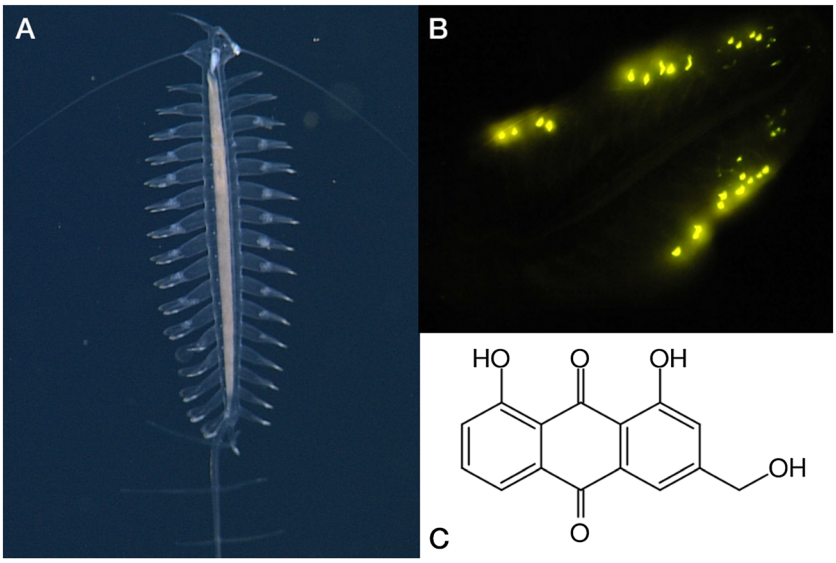

Figure 1. *Tomopteris* bioluminescence. (A) Photograph of a typical specimen, taken by the camera on the ROV. (B) Photo of the yellow bioluminescence, which begins at the tips of the parapodia and is then released into the water. The animal is oriented with the head at the bottom-left with the body axis up to the top-right. The camera was a NIKON D3 with ISO of 6400 and 10 s exposure time. Exposure time was longer than the luminescent emission to ensure capturing the event. Color is the natural color of luminescence as captured by the camera. (C) Chemical structure of aloe-emodin.

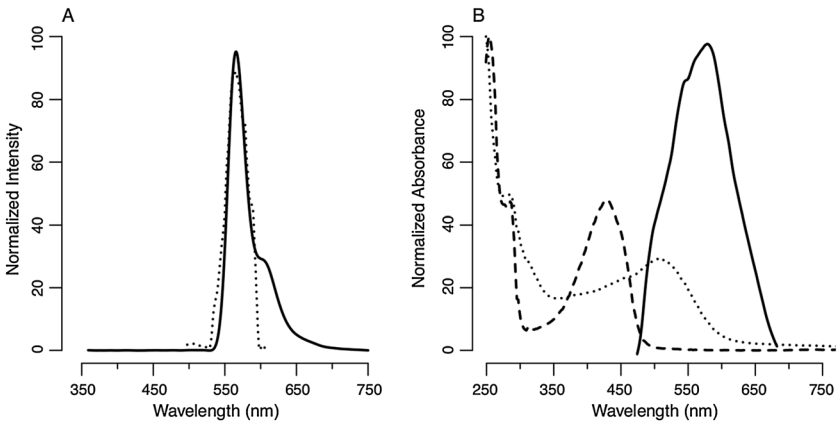

Figure 2. Various spectra. (A) The *in vivo* bioluminescence spectra of *Tomopteris* (solid line) and digitized *in vivo* fluorescence data from *Terio* (14) (dotted line). (B) Absorption spectra of the fluorescent pigment in methanol (dashed line) and with a drop of NaOH (dotted line), as well as the fluorescence emission spectrum in chloroform (solid line).

specimens and was seen even in specimens frozen for over 10 years. Owing to the irregularity of acquiring new specimens at sea and collecting exudate, we instead extracted material from frozen specimens (see Experimental).

Non-polar extractions

Frozen specimens were homogenized, and MeOAc was added to the homogenate. After centrifugation, nearly all of the fluorescent material was in the non-polar phase and appeared pale yellow. The absorption spectrum of the non-polar phase showed a large peak at 364 nm (Fig. S2). The aqueous layer was dimly fluorescent green, likely due to riboflavin or a similar compound. Often, the fluorescence of the MeOAc layer appeared bright yellow–green immediately after extraction (λ_{max}: 519 nm). When exposed to blue light, this changed to the characteristic yellow–orange color in seconds (Fig. S1B). This effect was attenuated in the presence of ascorbic acid, suggesting that oxygen or reactive oxygen species could be involved in this transition.

Purification of the yellow-orange compound by HPLC

This crude organic extract was separated by reversed-phase HPLC to isolate the fluorescent yellow–orange pigment (Fig. 3A). Very large absorption peaks at 254 and 430 nm of a yellow material with a bright fluorescence peak were observed around 5.6 min (Fig. 3B). This single peak was collected over multiple injections. The absorption (λ_{max}: 286, 430nm) and fluorescence emission (λ_{max}: 580, 548 nm shoulder) were acquired for the purified compound (Fig. 2B). The absorption peak of the purified compound is 430 nm, however, this does not appear to be abundant enough in the unpurified extract to show a distinct peak (Fig. S2). Instead, it likely that some other pigment accounts for the peak at 364 nm in the original MeOAc extract. Although the fluorescence emission

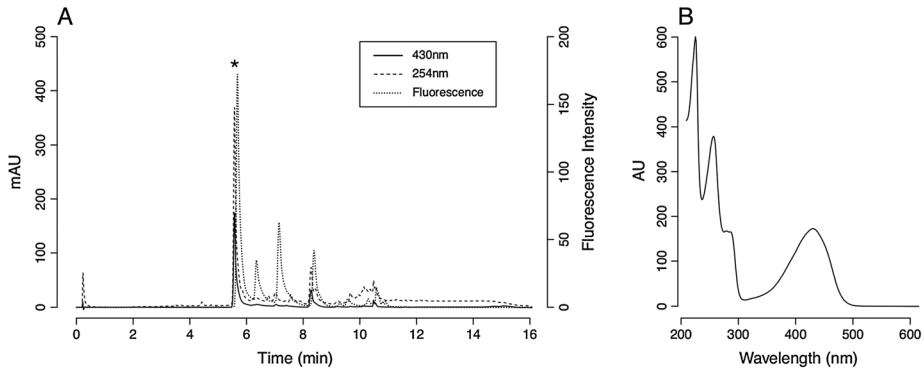

Figure 3. HPLC chromatogram of the MeOAc extract. (A) The UV/vis absorption (254 and 430 nm) and fluorescence chromatograms show a large peak of the fluorescent yellow–orange compound at 5.6 min, indicated by the star. (B) The corresponding absorption spectrum at 5.6 min clearly showing the characteristic peak at 430 nm.

does not perfectly match the digitized spectrum reported by Terio or the bioluminescence (Fig. 2B), (14) this may be due to the solvent or that the spectrum changes when bound by a protein, as seen for coelenterazine (17–19).

Mass determination and molecular formula

Knowing the absorption spectrum of the compound permitted easy mass determination of the compound with LCMS. The same MeOAc extract was analyzed by LCMS, where the yellow compound was identified at 337 *m/z* with the major fragment at 269 *m/z* (Fig. S3), corresponding to a mass difference of 68 *m/z*. To find the molecular formula and the identities of the fragments, the accurate mass was determined for the purified compound at 337.0331 *m/z*, corresponding to $C_{15}H_9O_5 + NaCHO_2$ (M-H). It was then determined that the major fragment was actually the molecular ion, at 269.0455 *m/z*, which indicated loss of the sodium formate adduct and the uncharged molecular formula of $C_{15}H_{10}O_5$ (Figs S4–S6).

Confirmation of the identity as aloe-emodin

The fluorescent material in methanol undergoes a bathochromic shift from yellow to red upon addition of saturated NaOH solution (Fig. 2B, dotted line, λ_{max}: 510 nm, also in Fig. 4).

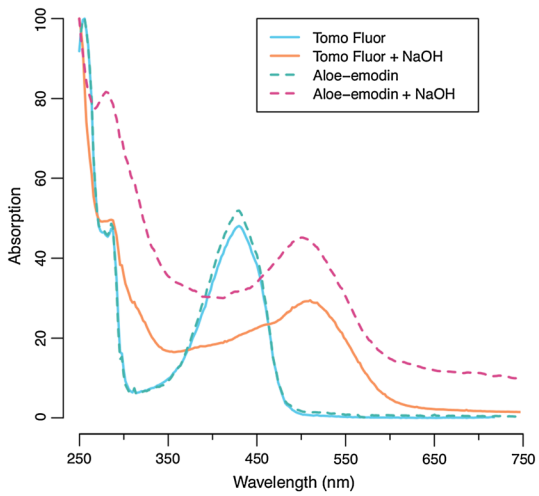

Figure 4. Spectra of aloe-emodin and the yellow–orange fluor. The measured absorption spectra of the purchased aloe-emodin and the yellow–orange fluor in methanol and with a drop of saturated NaOH solution.

The spectra and this transition are thought to be a property of 1,8-dihydroxy-9,10-anthraquinones (20). After comparison of our spectrum with 20 published UV/vis spectra of anthraquinones with the same molecular formula (21,22), we noticed that our spectrum is remarkably close to the reported spectrum of aloe-emodin (structure in Fig. 1C) (23). Aloe-emodin was purchased (Sigma-Aldrich) and was found to have an identical absorption spectrum as the yellow–orange fluor (Fig. 4).

The product ion mass spectrum (MS-MS) is sometimes used to confirm the presence of rare metabolites for cases where NMR cannot be used to deduce the structure (24). To ultimately confirm the identity of the compound, the HPLC-purified sample and a standard of aloe-emodin were sent out for analysis by LCMS. The retention time, the calculated and measured *m/z* ratios, and the product ion spectra were all identical matches, consistent with the hypothesis that the yellow–orange fluor is indeed aloe-emodin (Figs S7–S9).

Discussion

Extraction yield

Here, we described the extraction and identification of the yellow–orange fluor in the *Tomopteris*, which was first noted over 50 years ago. As the mass and structure were only determined towards the end of our experiments, some questions related to extraction yields were unaddressed. However, estimated from the published extinction coefficients of aloe-emodin, the HPLC data (from Fig. 3) suggest that the single injection of 10 μL contains in the order of 35 μg of aloe-emodin. Because multiple HPLC runs were necessary to separate all the material and not saturate the column, we estimate that even a relatively small worm (3–5 cm, estimated to be 200–500 mg) could contain 200 μg of aloe-emodin. Measurements of other *Tomopteris* specimens suggest dry material accounts for around 15% of the mass (25). For a 500 mg worm, this means that dry mass accounts for 75 mg, where 200 μg of aloe-emodin is almost a third of a percent of the dry mass.

Functions of quinones

We have ultimately confirmed the compound to be aloe-emodin, but we do not know the function of aloe-emodin for this marine animal. Given that aloe-emodin is an anthraquinone, it is logical that it is used similarly as other anthraquinones. There are a number of cases for insects in which quinones and anthraquinones have been suggested to have various defensive

roles, possibly as toxins (20,26,27). Quinones also are known to participate in redox reactions, such as in the electron transport chain. Because all known bioluminescence reactions involve an oxidation (15), quinones are well suited for this type of chemistry. Aloe-emodin has been discussed in literature for both antioxidant and prooxidant properties, making a strong case for its role in this regard (28–30).

Quinones in other bioluminescent systems

Furthermore, there is a precedent of a quinone in bioluminescence from an unusual polybrominated benzoquinone that is used in the luminous system of the acorn worm, *Ptychodera flava*, which also requires riboflavin (31,32). Given that the green color of the light of the acorn worm closely matches the fluorescence of riboflavin, it is possible that riboflavin is the light emitter and this benzoquinone serves as an electron carrier for the oxidation of riboflavin. Alternatively, the authors of that work had demonstrated that polybrominated quinones themselves were chemiluminescent, suggesting that perhaps riboflavin is only present as a fluor for resonant energy transfer to change the color of the emitted light.

Chemiluminescence of anthraquinones

Other anthraquinones have been shown to be chemiluminescent (λ_{max}: 568 nm) when reduced to the hydroquinone or semiquinone and reacted with molecular oxygen (33). Additionally, it was also shown that a semiquinone form was chemiluminescent (or fluorescent) in yellow–green (λ_{max}: 515 nm) (33). We hypothesize that aloe-emodin, a substituted anthraquinone, would have very similar properties. In fact, our observations of a fluorescent yellow–green compound which transitions to aloe-emodin (where it is fluorescent yellow–orange) suggest the possibility that the yellow–green compound is a reduced form of aloe-emodin, possibly the anthrone which would be very susceptible to oxidation (34,35). If aloe-emodin were the oxyluciferin in this context, then plausibly the fluorescent yellow–green compound, the anthrone or a similar compound, could be the luciferin.

Other discussions of *Tomopteris*

The only modern characterization of *Tomopteris* luminescence suggested that chemiluminescence could be elicited from homogenate with superoxide ions (6), as seen for several other polychaetes (36,37). A large amount of Triton-X (2%) was needed to solubilize the light-emitter, suggesting that the enzyme may be a membrane-bound photoprotein (6). However, we consider it is unlikely that the in vivo mechanism of light emission requires superoxide. For example, coelenterazine is chemiluminescent with superoxide yet the light output was an order of magnitude lower than the same quantity of coelenterazine bound to obelin and activated with calcium ions (38).

Theories of origins of aloe-emodin

It was surprising to find this compound in a deep-sea animal as it was discovered from several *Aloe* species. It is not known whether the *Tomopteris* synthesizes aloe-emodin or acquires it elsewhere, perhaps through its diet or from a symbiont. Many anthraquinones are biosynthesized through a convergent mechanism using polyketide synthases (39,40), a mechanism that is conserved across prokaryotes, fungi and plants, thus any of those modes of acquisition may be possible. A dietary link from land plants would be preposterous; however, there are other cases of anthraquinones from marine organisms, (41) including a marine fungus that lives commensally with a green alga and appears to produce several anthraquinones and an isomer of aloe-emodin (42). Another possibility is that a symbiont is generating the compound and there is some precedent for this scenario in metazoans. It was thought that some insects may synthesize their own polyketides (40), although one study had shown that the compounds were made by an uncultured bacterial symbiont (43). To our knowledge, there has not been a confirmed case of polyketide synthesis by metazoans. Although this does not rule out such a possibility, it suggests that the aloe-emodin from *Tomopteris* may ultimately derive from another organism or involve biosynthetic mechanisms other than polyketide synthases.

Conclusions

From our detailed purification and LCMS, we have shown that the fluorescent yellow–orange compound in Tomopteris is aloe-emodin. Evidence from the overlap of the fluorescence and bioluminescence spectra is very compelling to suggest that aloe-emodin is the final light-emitter for *Tomopteris* bioluminescence. While evidence from related systems favors the interpretation that aloe-emodin is the oxyluciferin, this does not exclude the possibility that aloe-emodin is an acceptor for resonant energy transfer from another molecule. Detailed chemical studies are needed to discern these two cases. Ultimately, full characterization of the *Tomopteris* luminous system may lead to a new generation of bioluminescent sensors or reporters, particularly for plants or fungi where many anthraquinones are endogenous.

Authors' contributions

WRF and SHDH designed experiments and analyzed data. WRF, MLP and SHDH caught animals. MLP and SHDH acquired the bioluminescence spectrum. WRF did the experiments. WRF wrote the paper with corrections from the other authors.

Acknowledgements

WRF would like to thank R Linington for helpful discussions and advice. The NIH National Institute of General Medical Sciences (ROI-GMO87198) to SHDH supported our work. This research was also supported by the David and Lucile Packard Foundation through the Monterey Bay Aquarium Research Institute. University of California-Santa Cruz LCMS facility was funded by NIH grant S10RR020939.

References

1. Hastings JW. Chemistries and colors of bioluminescent reactions: a review. Gene 1996;173:5–11.
2. Haddock SHD, Moline MA, Case JF. Bioluminescence in the sea. Ann Rev Mar Sci 2010;2:443–93.
3. Harvey EN. *Bioluminescence*. 1st edn. New York: Academic Press, 1952.
4. Harvey EN. Bioluminescence and fluorescence in the living world. Am J Physiol 1926;77:555–61.
5. Dales RP. Bioluminescence in pelagic polychaetes. J Fish Res Board Canada 1971;28:1487–9.
6. Shimomura O. *Bioluminescence: chemical principles and methods*. Singapore: World Scientific, 2006.

7. Shimomura O, Johnson FH. Calcium binding, quantum yield, and emitting molecule in *Aequorin* bioluminescence. Nature 1970;227(5265):1356–7.
8. Suzuki N, Sato M, Nishikawa K, Goto T. Synthesis and spectral properties of 2-(6′-hydroxybenzothiazol-2′-yl)-4-hydroxythiazole, a possible emitting species in the firefly bioluminescence. Tetrahedron Lett 1969;10:4683–4.
9. Suzuki N, Goto T. Firefly bioluminescence II. Identification of 2-(6′-hydroxybenzothiazol-2′-yl)-4-hydroxythiazole as a product in the bioluminescence of firefly lanterns and as a product in the chemiluminescence of firefly luciferin in DMSO. Tetrahedron Lett 1971;22:2021–4.
10. Gibson QH, Hastings JW. The oxidation of reduced flavin mononucleotide by molecular oxygen. Biochem J 1962;83:368–77.
11. Eley M, Lee J, Lhoste JM, Lee CY, Cormier MJ, Hemmerich P. Bacterial bioluminescence. Comparisons of bioluminescence emission spectra, the fluorescence of luciferase reaction mixtures, and the fluorescence of flavin cations. Biochemistry 1970;9:2902–8.
12. Towner RD, Neufeld HA, Shevlin PB. Some characteristics of riboflavin chemiluminescence. Arch Biochem Biophys 1970;137:102–8.
13. Terio B. Su un pigmento fluorescente presente nella pinna del remo dorsale di un annelide polichete (*Tomopteris septentrionalis* Steenstrup). Boll Soc Ital Biol Sper 1960;36:725–7.
14. Terio B. Possibili interrelazioni tra bioluminescenza e fluorescenza di materiali fotosensibili presenti nelle pinne e sui parapodi dei Tomopterid. Atti Soc Peloritana Sci Fis Mat Natur 1964;10:1–11.
15. Hastings JW. Biological diversity, chemical mechanisms, and the evolutionary origins of bioluminescent systems. J Mol Evol 1983;19:309–21.
16. Latz MI, Frank TM, Case JF. Spectral composition of bioluminescence of epipelagic organisms from the Sargasso Sea. Mar Biol 1988;98:441–6.
17. Hori K, Cormier MJ. Structure and synthesis of a luciferin active in the bioluminescent systems of sea pansy (*Renilla*) and certain other bioluminescent coelenterates. Chemilumin Biolumin. 1972: 361–8.
18. Shimomura O, Johnson FH. Chemical nature of bioluminescence systems in coelenterates. Proc Natl Acad Sci U S A 1975;72:1546–9.
19. Shimomura O, Teranishi K. Light-emitters involved in the luminescence of coelenterazine. Luminescence 2000;15:51–8.
20. Howard DF, Phillips DW, Jones TH, Blum MS. Anthraquinones and anthrones: occurrence and defensive function in a Chrysomelid beetle. Naturwissenschaften 1982;69(2):91–2.
21. Thomson RH. *Naturally occurring quinones*. 1st edn. New York: Academic Press; 1971.
22. Thomson RH. *Naturally occurring quinones III: recent advances*. 1st edn. London: Chapman and Hall, 1987.
23. Wamer WG, Vath P, Falvey DE. In vitro studies on the photobiological properties of aloe emodin and aloin A. Free Radical Biol Med 2003;34:233–42.
24. Lee DY, Bowen BP, Northen TR. Mass spectrometry-based metabolomics, analysis of metabolite–protein interactions, and imaging. Biotechniques 2010;49:557–65.
25. Clarke A, Holmes LJ, Gore DJ. Proximate and elemental composition of gelatinous zooplankton from the Southern Ocean. J Exp Mar Biol Ecol 1992;155:55–68.
26. Weatherston BJ. The chemistry of arthropod defensive substances. Quart Rev 1967;21:287–313.
27. Eisner T, Meinwald J. Defensive secretions of arthropods. Science 1966;153(3742):1341–50.
28. Malterud KE, Farbrot TL, Huse AE, Sund RB. Antioxidant and radical scavenging effects of anthraquinones and anthrones. Pharmacology 1993;47(Suppl 1):77–85.
29. Yen Gc. Antioxidant activity of anthraquinones and anthrone. Food Chem 2000;70:437–41.
30. Tian B, Hua Y. Concentration-dependence of prooxidant and antioxidant effects of aloin and aloe-emodin on DNA. Food Chem 2005;91:413–18.
31. Kanakubo A, Isobe M. Isolation of brominated quinones showing chemiluminescence activity from luminous acorn worm, *Ptychodera flava*. Bioorg Med Chem 2005;13:2741–7
32. Kanakubo A, Koga K, Isobe M, Yoza K. Tetrabromohydroquinone and riboflavin are possibly responsible for green luminescence in the luminous acorn worm, *Ptychodera flava*. Luminescence 2005;20:397–400.
33. Stauff J, Bartolmes P. Chemiluminescence on oxidative formation of triplet states of anthrasemiquinone- and anthraquinone-2-sulfonate. Ang Chem Int Ed 1970;9:307–8.
34. Foster H, Gardner JH. The preparation and hydrolysis of some polyhydroxyanthraquinone glucosides. J Am Chem Soc 1936;318:1934–6.
35. Hay JE, Haynes LJ. 605. The aloins. Part I. The structure of barbaloin. J Chem Soc 1956;3141–7.
36. Shimomura O, Johnson FH. *Chaetopterus* photoprotein: crystallization and cofactor requirements for bioluminescence. Science 1968;159(3820):1239–40.
37. Nicolas MT, Bassot JM, Shimomura O. Polynoidin: a membrane photoprotein isolated from the bioluminescent system of scale-worms. Photochem Photobiol 1982;35:201–7.
38. Lucas M, Solano F. Coelenterazine is a superoxide anion-sensitive chemiluminescent probe: its usefulness in the assay of respiratory burst in neutrophils. Anal Biochem 1992;206:273–7.
39. Bringmann G, Irmer A. Acetogenic anthraquinones: biosynthetic convergence and chemical evidence of enzymatic cooperation in nature. Phytochem Rev 2008;7:499–511.
40. Bringmann G, Noll TF, Gulder TaM, Grüne M, Dreyer M, Wilde C, et al. Different polyketide folding modes converge to an identical molecular architecture. Nat Chem Biology 2006;2:429–33.
41. Wolkenstein K, Schoefberger W, Müller N, Oji T. Proisocrinins A–F, brominated anthraquinone pigments from the stalked crinoid *Proisocrinus ruberrimus*. J Nat Prod 2009;72:2036–9.
42. Hawas UW, El-Beih AA, El-Halawany AM. Bioactive anthraquinones from endophytic fungus *Aspergillus versicolor* isolated from red sea algae. Arch Pharm Res 2012;35:1749–56.
43. Piel J. A polyketide synthase-peptide synthetase gene cluster from an uncultured bacterial symbiont of *Paederus* beetles. Proc Natl Acad Sci U S A 2002;99:14002–7.

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web site.