

TRACE CHARACTERIZATION OF THE FLUORESCENT SUBSTANCES
OF A DINOFLAGELLATE, NOCTILUCA MILIARIS

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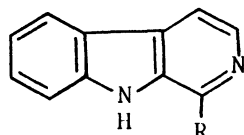
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Two fluorescent substances isolated from Noctiluca miliaris were identified as harman and norharman which were first examples isolated from marine sources.

The marine dinoflagellate Noctiluca miliaris, often responsible for red tides having a weak toxicity, is well known to show a brief flash of luminescence in coastal waters throughout the world. A large number of studies have been done for the establishment of the luminescent systems of the unicellular dinoflagellates such as Gonyaulax polyedra and Noctiluca miliaris,¹⁾ but the structure of any chemical substances related to the luminescence has been left unsolved. Fogel and Hastings²⁾ extracted an unstable luciferin from G. polyedra, but no structural information was reported. An approach to solve this problem is to search fluorescent substances in the organisms, because some fluorescent compound must be produced from the luciferin during its luminescence.³⁾ Indeed, Eckert⁴⁾ showed that both bioluminescence and fluorescence were observed in the same subcellular inclusions in a cell of Noctiluca.

Now, we wish to report here that two major fluorescent substances isolated from Noctiluca miliaris were identified as harman (I) and norharman (II), whose characterization from marine sources has not been reported in the literatures.



I : R=Me

II : R=H

A frozen slurry of Noctiluca⁵⁾ in sea water (1.8 kg) was homogenized gently (ca 5000 rpm) until the temperature was allowed to rise to 0°C, and then centrifuged, when about 15 g (wet weight) of broken cells⁶⁾ were precipitated. The supernatant was lyophilized, the residue (ca 50 g) was extracted with MeOH (500 ml) and the extract was concentrated to almost dryness. Water (20 ml) was added to the residue, and the resulting solution (pH ~3) was basified with NaHCO₃ and extracted five times with ether. From the ether extract, basic substances were isolated as usual

and purified by successive silica gel TLC [solvents: EtOAc-MeOH-H₂O (30:1:1) and MeOH-CH₂Cl₂ (1:10)] to give several fluorescent compounds. Two major substances, I [40 µg,⁷⁾ m/e 182 (M⁺), Rf on SiO₂ TLC: 0.39 in EtOAc-MeOH-H₂O (30:1:1)] and II [5 µg,⁷⁾ m/e 168 (M⁺), Rf on SiO₂ TLC: 0.43 in EtOAc-MeOH-H₂O (30:1:1)], showed characteristic UV spectra and were identified as harman and norharman by comparison of physical data (Rf values on TLC and HPLC, UV, mass and FT-PMR spectra) with those of the authentic specimens. Whether I and II are related to the luminescent system in Noctiluca is not yet known and its clarification needs further investigation.⁸⁾

An additional interest in the first characterization of I and II in Noctiluca is focused on their toxicity⁹⁾ in relation to toxic red tides. The investigation on the role of harman and norharman in Noctiluca and also on the characterization of other fluorescent substances is in progress.

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- 5) The material was collected by plankton net from the red tides at Mikawa-Bay in spring, 1979. The similar results were obtained from red tides at Ise-Bay (1978) and Osaka-Bay (1977).
- 6) I and II were also detected in the extract of the cells.
- 7) The amounts of I and II were calculated from the O.D. in their UV spectra.
- 8) Fluorescent spectra of I and II: λ_{\max} 432 nm (pH 6.0); Luminescent spectra of Noctiluca miliaris: λ_{\max} 475 nm (pH 6.0).
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