

Supporting Information

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Investigation of Insect Repellent Activity of Cyclocolorenone obtained from the Red Alga *Laurencia intricata*

Takahiro Ishii*¹, Yuto Shinjo¹, Miyu Miyagi¹, Hiroshi Matsuura²,
Tsuyoshi Abe³, Norio Kikuchi⁴ and Minoru Suzuki⁴

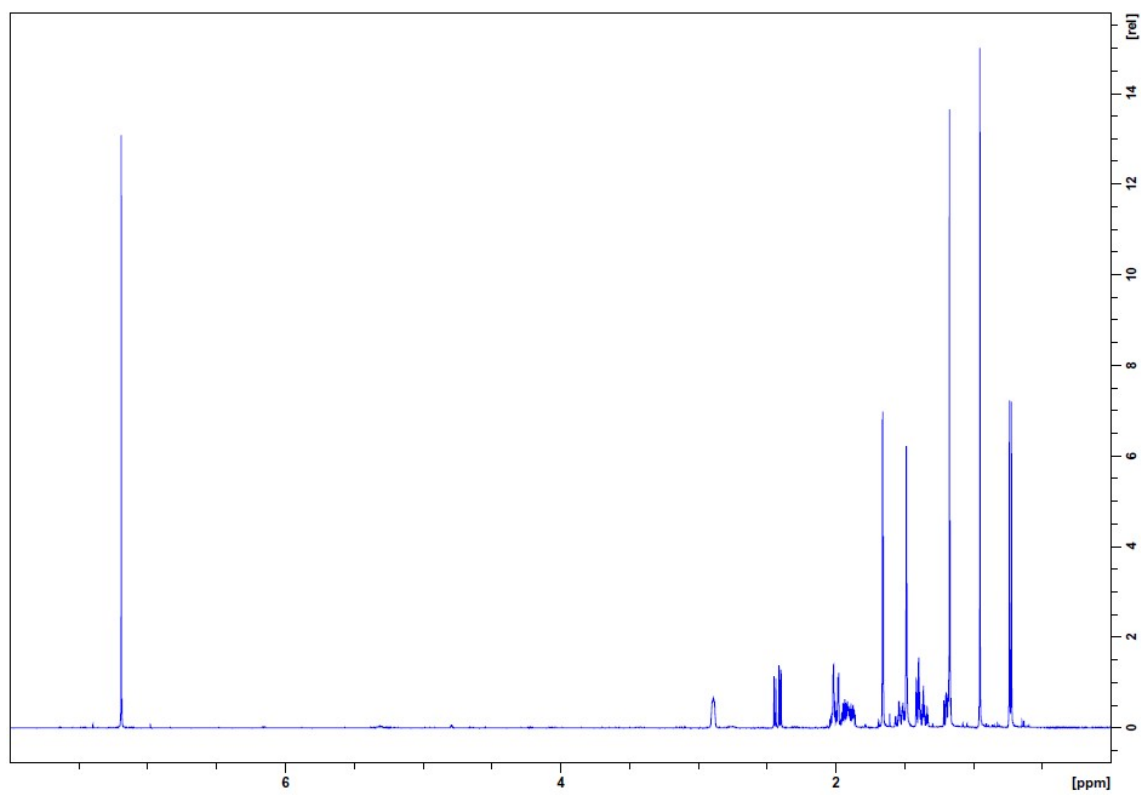
¹*Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*

²*Department of Materials Chemistry, National Institute of Technology, Asahikawa College, 2-2-1-6 Shunkodai, Asahikawa 071-8142, Japan*

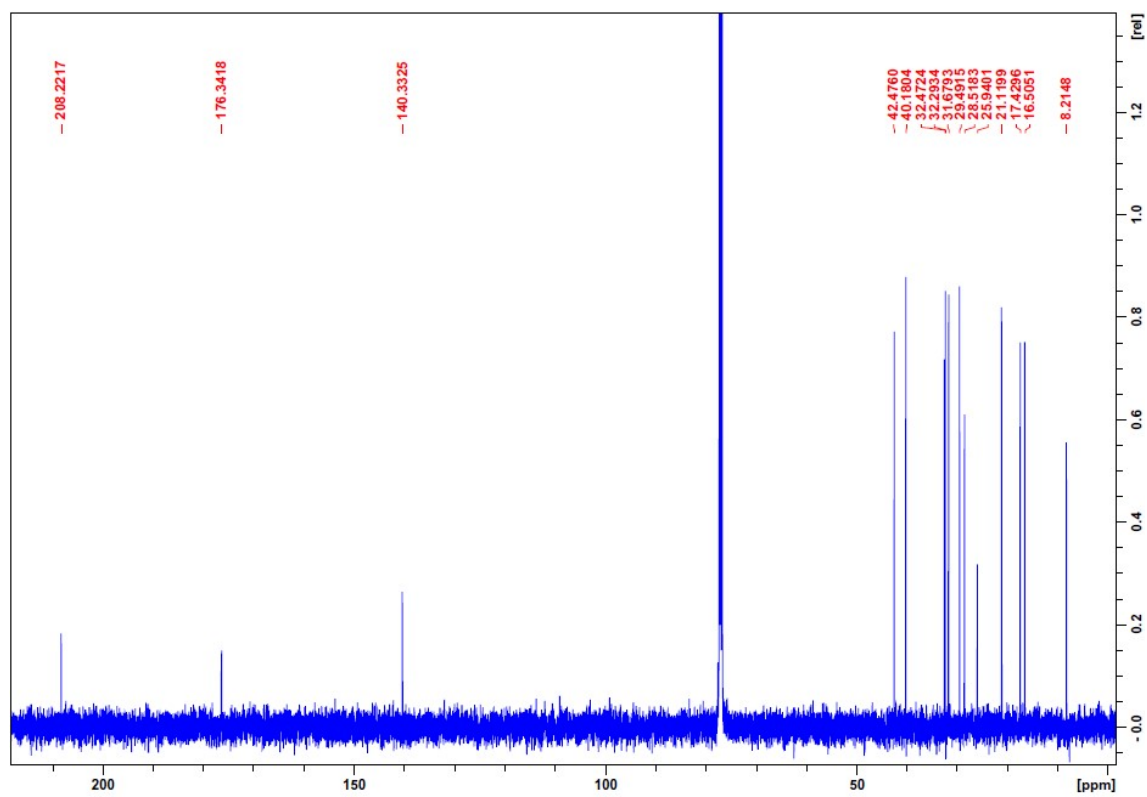
³*The Hokkaido University Museum, Hokkaido University, Sapporo 060-0810, Japan*

⁴*Coastal Branch of Natural History Museum and Institute, Chiba, 123 Yoshio, Katsuura, Chiba 299-5242, Japan*

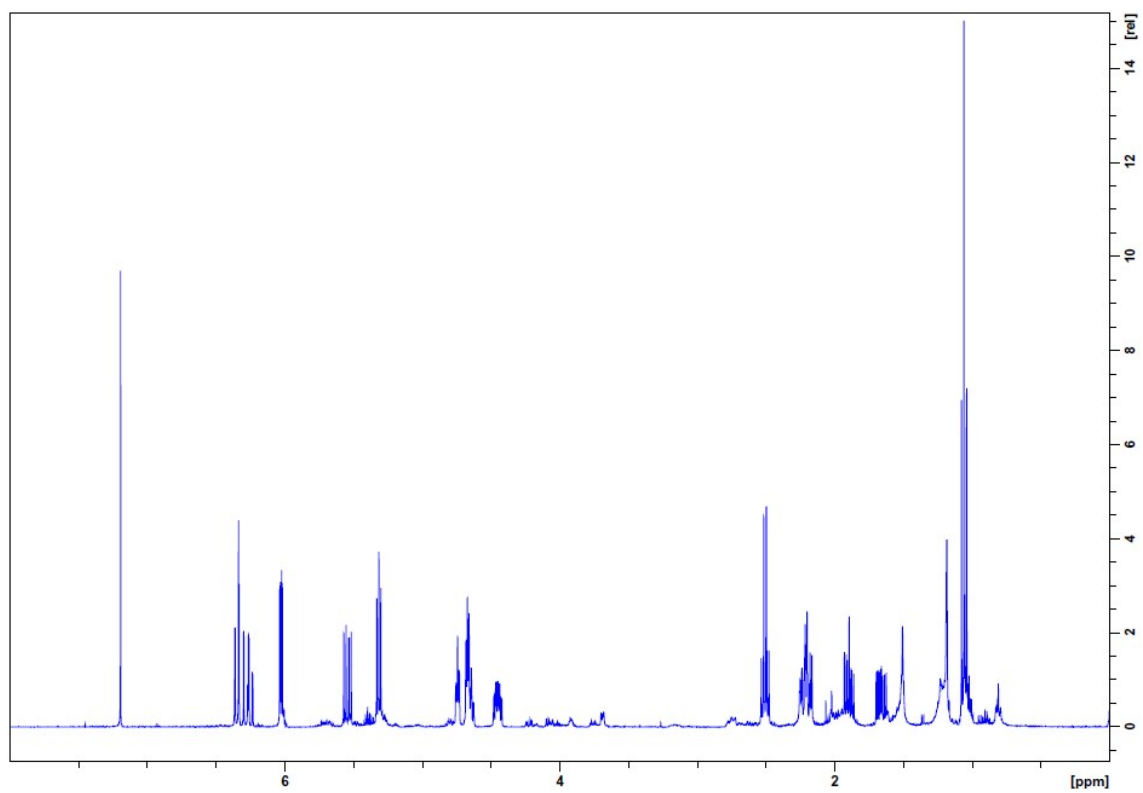
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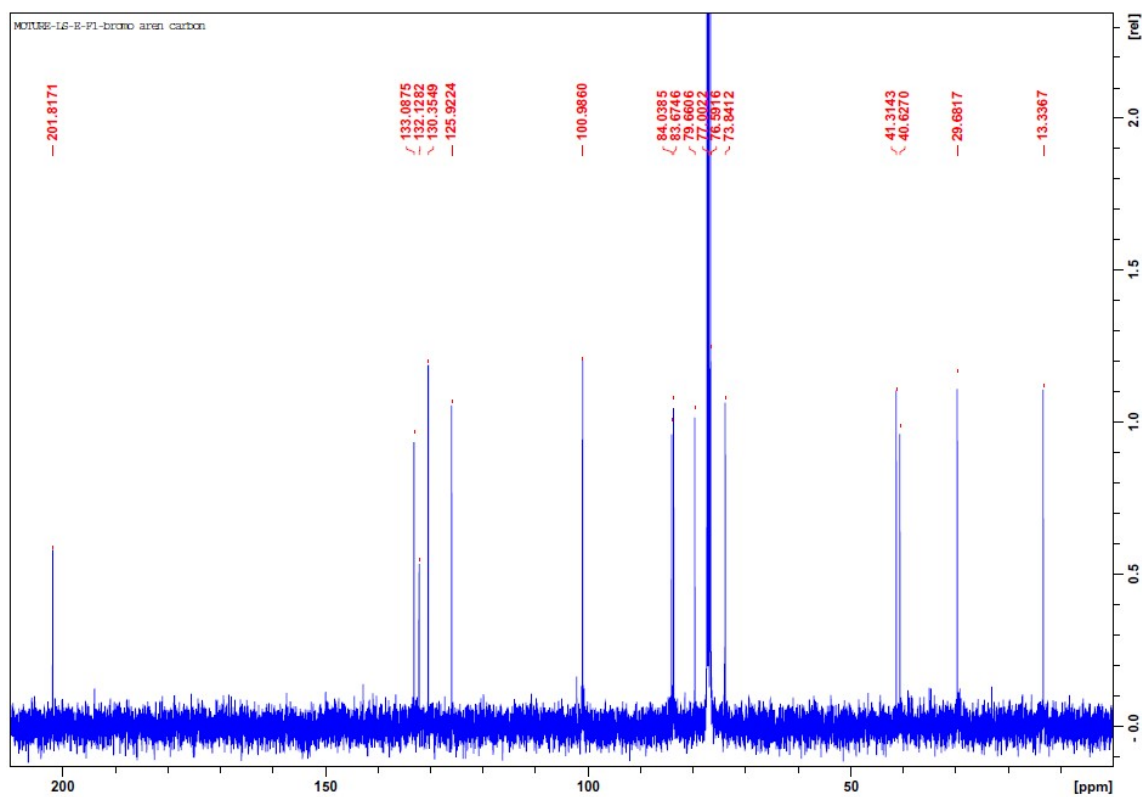
S1: ¹H-NMR (400 MHz, CDCl₃) spectrum of cyclocolorone (**1**)



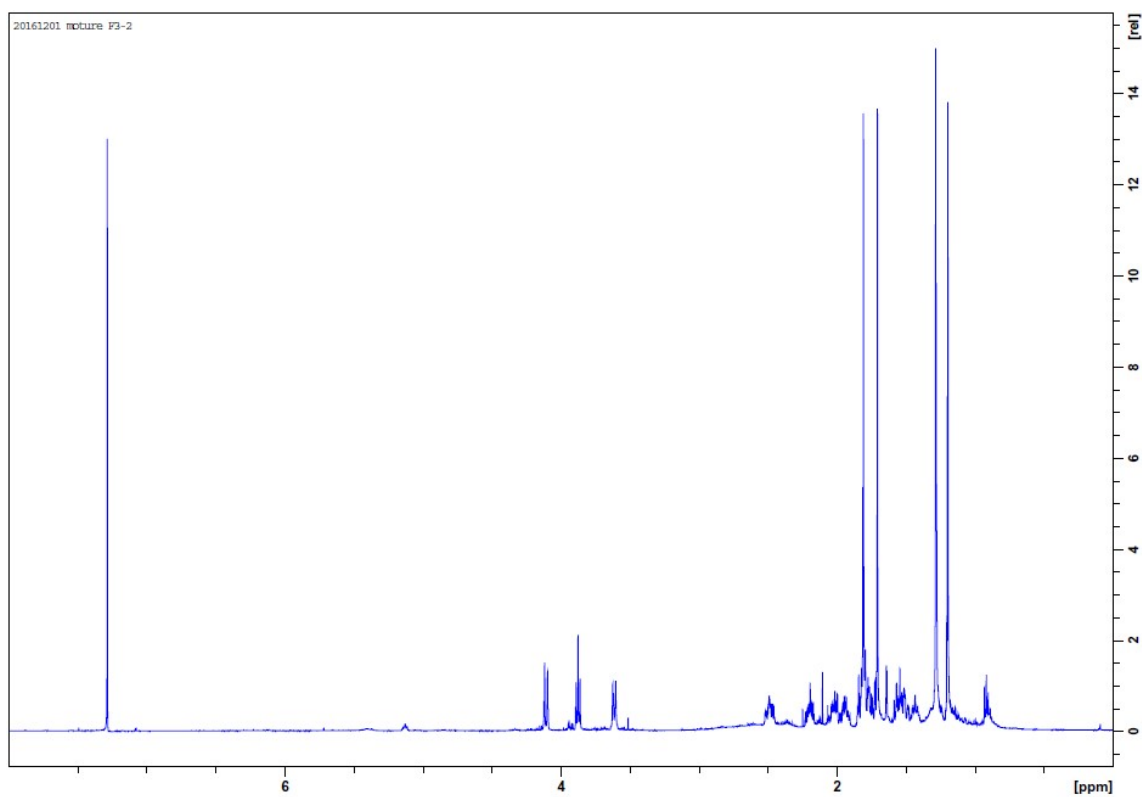
S2: ^{13}C -NMR (100 MHz, CDCl_3) spectrum of cyclocolorone (1)



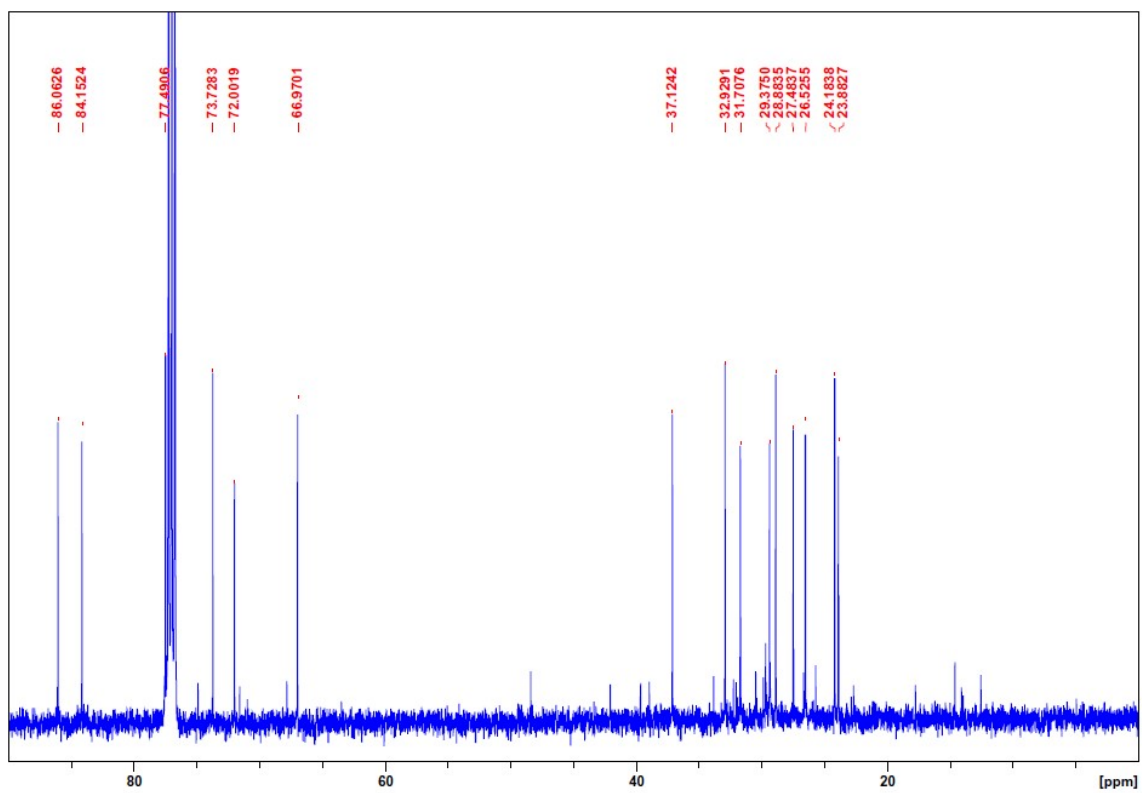
S3: ¹H-NMR (400 MHz, CDCl₃) spectrum of zagashimallene (**2**)



S4: ¹³C-NMR (100 MHz, CDCl₃) spectrum of zagashimallene (**2**)



S5: $^1\text{H-NMR}$ (400 MHz, CDCl_3) spectrum of intricatetraol (**3**)



S6: ^{13}C -NMR (100 MHz, CDCl_3) spectrum of intricatetraol (3)

S7. Materials and Methods

7.1. General

Optical rotations were measured on a Jasco P-1010 polarimeter. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AVANCE III Nanobay spectrometer. ESI-MS spectra were obtained on a Waters Xevo TQD spectrometer. Semi-preparative HPLC was performed on a Shimadzu HPLC system (CBM-20A system controller, LC-20AT binary pump, and SPD-20A UV/VIS detector) with Luna 5μ C18(2) 100A column (250 x 10 mm I.D.). Preparative TLC was performed with silica gel plates (Merck, Kieselgel 60 F₂₅₄). Silica gel (Merck, Kieselgel 60, 70–230 mesh) was used for column chromatography. Analytical TLC was performed on Merck Kieselgel 60 F₂₅₄. Spots were visualized by UV light and/or spraying with a 5% phosphomolybdic acid-ethanol solution followed by heating at 100°C.

7.2. Algal Material

The red alga *Laurencia intricata* was collected off the coasts of Katsuura, Chiba, Japan on May 24, 2016. A voucher specimen (CMNH-BA-007526) was deposited in the Coastal Branch of Natural History Museum and Institute, Chiba.

7.3. Extraction and Isolation

The dried alga (26.9 g) was homogenized in MeOH and then extracted twice with MeOH (250 mL) at room temperature for 24 h. After filtration, the crude extract was evaporated under reduced pressure and then partitioned between EtOAc and H₂O. The EtOAc solution was washed with H₂O, dried (anhydrous Na₂SO₄) and evaporated to leave a dark green oil (697.5 mg). A portion (543.5 mg) of the extract was fractionated by Si-gel column chromatography with a step gradient (CHCl₃ and MeOH). A portion (35.6 mg) of the fraction (240.8 mg) eluted with CHCl₃ was further separated by preparative TLC with toluene to yield compound **2** (12.8 mg). A portion (35.2 mg) of the fraction (136 mg) eluted with CHCl₃/MeOH (99:1) was further separated by a combination of preparative TLC with *n*-hexane/EtOAc (3:1) and HPLC (Luna 5μ C18(2) 100A) with MeCN to afford compound **1** (2.2 mg). In addition, a portion (38 mg) of the fraction (144.5 mg) eluted with CHCl₃/MeOH (95:5) was submitted to preparative TLC with *n*-hexane/EtOAc (2:1) to give compound **3** (6.3 mg).

7.4. Insect Repellent Bioassay

The repellent activities of all isolated compounds against the maize weevils *Sitophilus zeamais* were evaluated using the filter paper impregnation method, as previously described [11]. The numbers of adult beetles present in each Petri dish were recorded after 24 h of exposure. Each treatment was repeated three times. Pyrethrin standard was used as a positive control.