Supplementary Material for

Rearranged Terpenoids from the Marine Sponge *Darwinella* cf. *oxeata* and its Predator, the Nudibranch *Felimida grahami*

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1. Detailed Isolation Procedure for Compounds **2** – **7**.

Specimens of *D. oxeata* were removed from the EtOH (300 mL) and extracted with MeOH (3 x 300 mL, 30 min in an utrasound bath). The extracts were pooled and evaporated. The resulting organic extract was suspended in 95% MeOH and partitioned with hexane (3 x 200 mL). After evaporation, the MeOH extract was suspended in H_2O and extracted with EtOAc (3 x 500 mL). The EtOAc extract was evaporated to give 1.79 g of crude material.

The EtOAc fraction (1.79 g) was fractionated by Sephadex LH20 (MeOH) column chromatography, to give six fractions: E1A-1 (385.6 mg), E1A-2 (178.1 mg), E1A-3 (257.5 mg), E1A-4 (511.5 mg), E1A-5 (153.2 mg) and E1A-6 (72.9 mg). The fractions were analyzed by HPLC-UV-MS, using an analytical C_{18} reversed-phase column (Waters X-Terra MS C_{18}, 3.5 μm, 2.1 x 50 mm) with a linear gradient of 1:1 MeOH/MeCN in H_2O (with 0.1% HCO_2H) as eluent, starting at 80% to 0% H_2O over 22 min, at a flow rate of 1.0 mL/min. Detection was monitored by UV between λ_{max} 200 and 400 nm and by positive ion ESIMS with a cone voltage of 25 V monitoring ions between *m/z* 180 and 700. Fractions E1A-3 and E1A-4 were pooled (769.0 mg) and subjected to a solid-phase extraction on a C_{18} reversed-phase silica-gel cartridge (10 g) eluted with 40:60, 50:50, 60:40, 70:30, 90:10 MeOH/H_2O and 100% MeOH. Six fractions were obtained: E1A-34-1 (153.4 mg), E1A-34-2 (165.4 mg), E1A-34-3 (190.9 mg), E1A-34-4 (132.9 mg), E1A-34-5 (71.2 mg) and E1A-34-6 (15.0 mg), which were analyzed by HPLC-UV-ESIMS. Fraction E1A-34-1 was further fractionated by reversed-phase HPLC (Inertsil ODS-2 SP, 5 μm, 7.6 x 250 mm, GL Sciences Inc.) using an isocratic elution with 76:24 MeOH/H_2O and 0.1% HCO_2H as eluent over 30 min, at 1.5 mL/min. Five fractions were obtained: E1A-34-11 (59.0 mg), E1A-34-12 (13.6 mg), E1A-34-13 (27.2 mg), E1A-34-14 (2.8 mg) and E1A-34-15 (4.1 mg), which were analyzed by HPLC-UV-MS. Fraction E1A-34-13 was fractionated by HPLC using a C_8 reversed-phase column (Inertsil C_8-4, 5 μm, 4.6 x 250 mm, GL Sciences Inc.) using a gradient elution from 70% MeOH/H_2O to 75% MeOH/H_2O with 0.1% HCO_2H as eluent over 15 min, at 0.8 mL/min, to give two fractions: E1A-34-13-A (5.9 mg) and E1A-34-13-B (1.3 mg). Fraction E1A-34-13-A was purified by HPLC using a C_8 reversed-phase column (Inertsil C_8-4, 5 μm, 4.6 x 250 mm, GL Sciences Inc.) and MeCN/H_2O 35:65 as eluent over 20 min, at 1.0 mL/min to give a pure sample of oxeatamide A (**6**, 4.0 mg). Fraction E1A-34-13-B was purified by HPLC using a C_{18} Inertsil ODS-EP column (5 μm, 4.6 x 250 mm) and MeCN/H_2O 45:55 as eluent over 20 min, at 1.0 mL/min, to give oxeatamide A methyl ester (**7**, 0.5 mg). Fraction E1A-34-2 (165.4 mg) was fractionated by reversed-phase HPLC (Inertsil ODS-3,

5 μm, 4.6 x 250 mm, GL Sciences Inc.) using a linear gradient from 80% MeOH/H_2O to 88% MeOH/H_2O with 0.1% HCO_2H as eluent over 25 min, at 1.0 mL/min, to give five fractions E1A-34-21 (49.6 mg), E1A-34-22 (24.3 mg), E1A-34-23 (38.1 mg), E1A-34-24 (30.4 mg) and E1A-34-25 (28.1 mg), which were analyzed by HPLC-UV-MS. Fraction E1A-34-23 was purified by HPLC using a C_{18} Inertsil ODS-3 column (5 μm, 4.6 x 250 mm) and MeOH/MeCN/H_2O 20:40:40 with 0.1% HCO_2H as eluent over 30 min, at 1.0 mL/min, to give four fractions: E1A-34-23-A (2.6 mg), E1A-34-23-B (9.7 mg), E1A-34-23-C (2.1 mg) and E1A-34-23-D (6.6 mg). Fraction E1A-34-23-B was purified by HPLC using a C_8 reversed-phase column (Inertsil C_8-4, 5 μm, 4.6 x 250 mm, GL Sciences Inc.) and MeCN/H_2O 35:65 with 0.1% HCO_2H as eluent over 20 min, at 1.0 mL/min, to give an additional amount of **6** (4.4 mg). Fraction E1A-34-23-D was purified by HPLC using a C_{18} Delta-Pak™ SP column (15 μm, 7.8 x 300 mm, Waters) and MeCN/H_2O 55:45 with 0.1% HCO_2H as eluent over 20 min, at 1.8 mL/min, to give pure sample of oxeatamide A methyl ester (**7**, 1.6 mg). Fraction E1A-34-24 was purified by HPLC using a C_{18} X-Terra column (5 μm, 4.6 x 250 mm) and MeCN/H_2O 40:60 with 0.1% HCO_2H as eluent over 40 min, at 1.0 mL/min, to give nine fractions: E1A-34-24-A (0.8 mg), E1A-34-24-B (1.4 mg), E1A-34-24-C (0.8 mg), E1A-34-24-D (1.7 mg), E1A-34-24-E (5.7 mg), E1A-34-24-F (2.7 mg), E1A-34-24-G (2.9 mg), E1A-34-24-H (4.9 mg) and E1A-34-24-I (7.5 mg). Fraction E1A-34-24-B was identified as oxeatamide H (**3**, 1.4 mg). Fraction E1A-34-25 was separated by HPLC using a C_{18} X-Terra column (5 μm, 4.6 x 250 mm) and MeCN/H_2O 50:50 with 0.1% HCO_2H as eluent over 30 min, at 1.0 mL/min, to give fraction E1A-34-25-A (5.7 mg) that was purified by HPLC using a C_{18} X-Terra column (5 μm, 4.6 x 250 mm) and MeOH/H_2O 70:30 with 0.1% HCO_2H as eluent over 15 min, at 1.0 mL/min, to give oxeatine (**2**, 1.7 mg). Fraction E1A-34-3 was purified by HPLC using a C_{18} Delta-Pak SP column (15 μm, 7.8 x 300 mm, Waters) using gradient elution from 80% to 90% MeCN/H_2O with 0.1% HCO_2H as eluent over 30 min, at 1.5 mL/min, to give five fractions: E1A-34-31 (4.1 mg), E1A-34-32 (15.1 mg), E1A-34-33 (51.1 mg), E1A-34-34 (65.8 mg) and E1A-34-35 (31.4 mg). Fraction E1A-34-33 was fractionated by HPLC using a C_{18} Delta-Pak™ SP column (15 μm, 7.8 x 300 mm, Waters) and MeCN/H_2O 65:35 with 0.1% HCO_2H as eluent over 30 min, at 1.5 mL/min, to give six fractions: E1A-34-33-A (2.1 mg), E1A-34-33-B (9.8 mg), E1A-34-33-C (3.7 mg), E1A-34-33-D (5.2 mg), E1A-34-33-E (14.5 mg) and E1A-34-33-F (2.8 mg). Fraction E1A-34-33-E was purified by HPLC using a C_{18} X-Terra column (5 μm, 4.6 x 250 mm) and MeCN/H_2O 45:55 with 0.1% HCO_2H as eluent over 20 min, at 1.2 mL/min, to give membranolide (**1**, 9.6 mg). Fraction E1A-34-4 was separated by reversed-phase HPLC (Inertsil ODS-2 SP, 5 μm, 7.6 x 250 mm, GL Sciences Inc.) using an isocratic elution of 60:40

$MeCN/H_2O$ with 0.1% HCO_2H as eluent over 30 min, at 1.5 mL/min. Five additional fractions were obtained: E1A-34-41 (3.8 mg), E1A-34-42 (2.0 mg), E1A-34-43 (3.7 mg), E1A-34-44 (3.1 mg) and E1A-34-45 (13.1 mg). Fraction E1A-34-42 was purified by HPLC using a C_{18} X-Terra column (5 µm, 4.6 x 250 mm) and $MeOH/H_2O$ 50:50 with 0.1% HCO_2H as eluent over 20 min, at 1.0 mL/min, to give an additional amount (1.2 mg) of membranolide (**1**). Fractions E1A-5 and E1A-6 were pooled (225.0 mg) and were fractionated by reversed-phase HPLC (Inertsil ODS-2 SP, 5 µm, 7.6 x 250 mm, GL Sciences Inc.) using gradient elution from 10% to 50% $MeOH/H_2O$ with 0.1% HCO_2H as eluent over 40 min, at 1.5 mL/min, to give eight fractions: E1A-56-1 (15.2 mg), E1A-56-2 (23.7 mg), E1A-56-3 (11.5 mg), E1A-56-4 (36.1 mg), E1A-56-5 (31.6 mg), E1A-56-6 (22.5 mg), E1A-56-7 (10.3 mg) and E1A-56-8 (59.8 mg). These fractions were analyzed by HPLC-UV-ESIMS and SciFinder and MarinLit databases were used for dereplication. Fraction E1A-56-8 was purified by HPLC using a C_8 reversed-phase column (Inertsil C_8-4, 5 µm, 4.6 x 250 mm, GL Sciences Inc.) and $MeCN/H_2O$ 50:50 with 0.1% HCO_2H as eluent over 45 min, at 3.0 mL/min, to give eight fractions: E1A-56-81 (14.8 mg), E1A-56-82 (8.8 mg), E1A-56-83 (10.1 mg), E1A-56-84 (0.9 mg), E1A-56-85 (2.8 mg), E1A-56-86 (3.1 mg), E1A-56-87 (1.1 mg) and E1A-56-88 (3.4 mg). Fraction E1A-56-82 was purified by HPLC using a C_{18} InertSustain column (5 µm, 4.6 x 250 mm, GL Sciences) and $MeCN/H_2O$ 40:60 as eluent over 40 min, at 1.0 mL/min, to give four fractions: E1A-56-82-A (0.7 mg), E1A-56-82-B (1.7 mg), E1A-56-82-C (1.8 mg) and E1A-56-82-D (0.5 mg). Fraction E1A-56-82-C was purified by HPLC using a C_{18} X-Terra column (5 µm, 4.6 x 250 mm) with a linear gradient of 1:1 MeOH/MeCN in H_2O (with 0.1% HCO_2H), starting at 90% to 0% H_2O over 40 min, at a flow rate of 1.0 mL/min, to give oxeatamide I (**4**, 1.2 mg). Fraction E1A-56-85 was purified by HPLC using a C_{18} X-Terra column (5 µm, 4.6 x 250 mm) with a linear gradient of 1:1 MeOH/MeCN in H_2O (with 0.1% HCO_2H), starting at 90% to 0% H_2O over 40 min, at a flow rate of 1.0 mL/min, to give oxeatamide J (**5**, 2.3 mg).

2. Schemes of Isolation Procedures for Compounds **2** – **5**.

The fraction EtOAc (1.7925 g, see Experimental Section) was separated by a series of chromatographic steps summarized in Figure S1. The specific conditions for the separations steps 1-10 are the following:

1. Sephadex LH20 (MeOH).

2. Column chromatography C18 reversed phase gradient MeOH in H_2O from 60:40 to 100% MeOH

3. HPLC condition 1: C_{18} reversed-phase column (Inertsil ODS-3, 5 µm, 4.6 x 250 mm, GL Sciences Inc.) linear gradient of 80% to 88% MeOH/H_2O with 0.1% formic acid over 25 min; flow rate: 1.0 mL/min.

4. HPLC condition 2: C_{18} X-Terra column (5 µm, 4.6 x 250 mm) isocratic 40:60 MeCN/H_2O with 0.1% formic acid over 40 min; flow rate: 1.0 mL/min.

5. HPLC condition 3: C_{18} X-Terra column (5 µm, 4.6 x 250 mm) isocratic 1:1 MeCN/H_2O with 0.1% formic acid over 30 min; flow rate: 1.0 mL/min.

6. HPLC condition 4: C_{18} X-Terra column (5 µm, 4.6 x 250 mm) isocratic 70:30 MeOH/H_2O with 0.1% formic acid over 15 min; flow rate: 1.0 mL/min.

7. HPLC condition 5: C_{18} reversed-phase column (Inertsil ODS-2 SP, 5 µm, 7.6 x 250 mm, GL Sciences Inc.) gradient of 10% to 50% MeOH/H_2O with 0.1% formic acid over 40 min; flow rate: 1.5 mL/min.

8. HPLC condition 6: C_8 reversed-phase column (Inertsil C8-4, 5 µm, 4.6 x 250 mm, GL Sciences Inc.) isocratic 1:1 MeCN/H_2O with 0.1% formic acid over 45 min; flow rate: 3.0 mL/min.

9. HPLC condition 7: C_{18} InertSustain column (5 µm, 4.6 x 250 mm, GL Sciences) isocratic 40:60 MeCN/H_2O over 40 min; flow rate: 1.0 mL/min.

10. HPLC condition 8: C_{18} X-Terra column (5 µm, 4.6 x 250 mm) linear gradient of 1:1 MeOH/MeCN in H_2O (with 0.1% formic acid), starting at 90% until 0% H_2O over 40 min; flow rate: 1.0 mL/min.

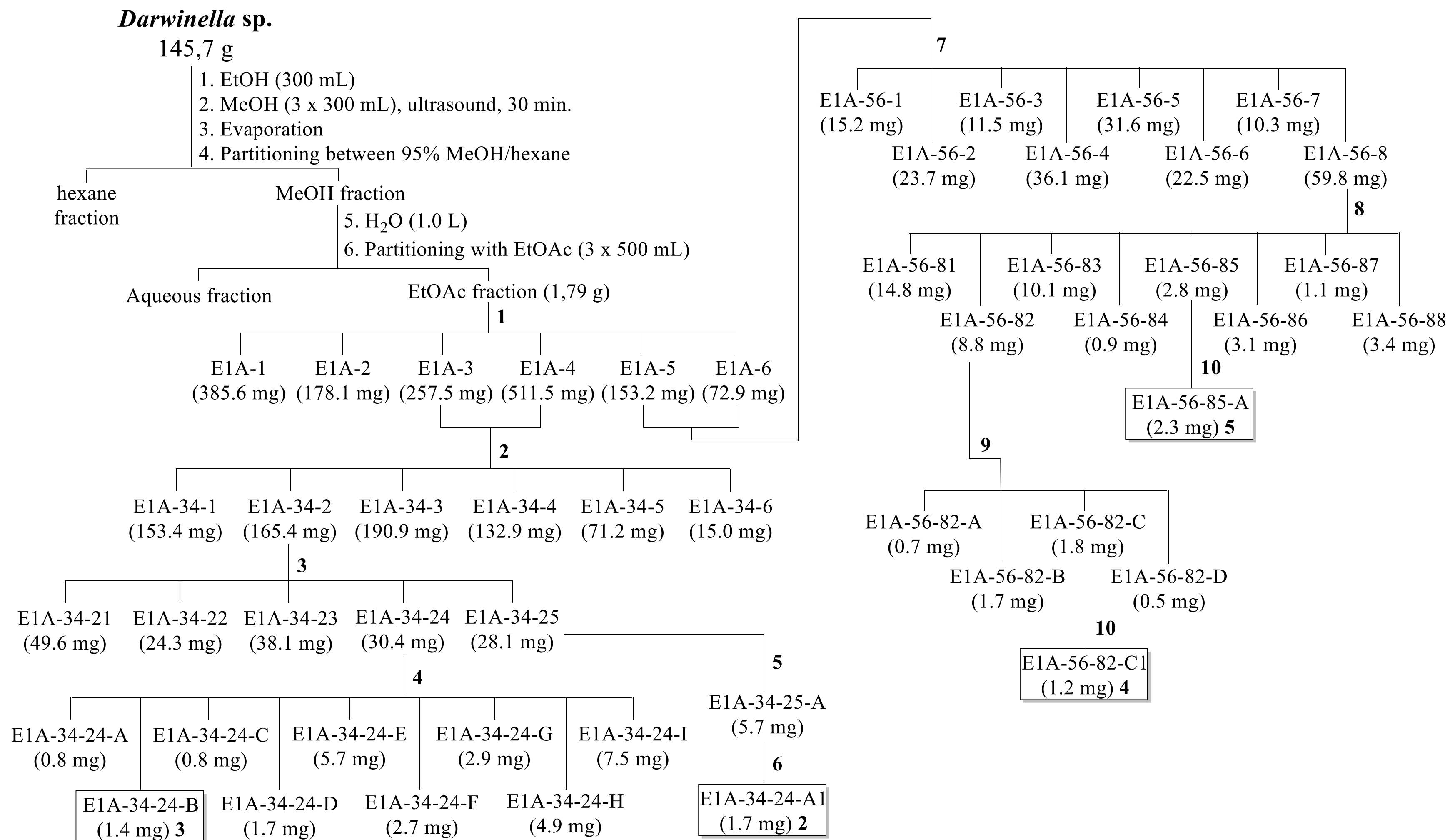

Figure S1. Separation scheme for the isolation of oxeatine (**2**) and oxeatamides H – J (**3** – **5**).

3. Procedure for the anti-*Trypanosoma cruzi* bioassay

BALB/c mice were obtained by the animal breeding facility at the Adolfo Lutz Institute-SP, Brazil. The animals were maintained in sterilized cages under a controlled environment and received water and food *ad libitum*. Animal procedures were performed with the approval of the Research Ethics Commission (project CEUA/IAL-Pasteur 02/2011).

Trypomastigotes of *Trypanosoma cruzi* (Y strain) were maintained in Rhesus monkey kidney cells (LLC-MK2-ATCC CCL 7), cultivated in RPMI-1640 medium supplemented with 2% FBS at 37 °C in a 5% CO_2-humidified incubator. Macrophages were collected from the peritoneal cavity of BALB/c mice by washing them with RPMI-1640 medium supplemented with 10% FBS and were maintained at 37 °C in a 5% CO_2-humidified incubator. The murine fibroblasts (NCTC clone 929, ATCC) were maintained in RPMI-1640 supplemented with 10% FBS at 37 °C in a humidified atmosphere containing 5% CO_2.

In order to determine the 50% inhibitory concentration (IC_{50}) against *T. cruzi*, trypanosomes were obtained from LLC-MK2 cultures, seeded at $1x10^6$ cells/well in 96-well plates and incubated with samples (maximal concentration of 100 µM) during 24 h at 37 °C in a 5% CO_2-humidified incubator. The viability was determined by determined by the resazurin assay (0.011% in PBS).[1]

The activity against intracellular parasites was determined in infected macrophages. Macrophages obtained as previously described and seeded for 24 h at $1x10^5$ cells/well in 16-well slide chambers (Nunc). Trypomastigotes of *T. cruzi* obtained from LLC-MK2 cultures were added to the macrophages at a ratio of 1:5 (macrophage/parasite) for 4 h at 37 ºC in 5% CO_2 atmosphere. Non-internalized parasites were removed by washing once with medium and the cells were then incubated with the test samples (maximal concentration of 55 µM) during 48 h for *T. cruzi* treatment at 37 ºC in 5% CO_2 atmosphere. Benznidazol were used as the standard drug control. At the end of the assay, the cells were fixed in MeOH, stained with Giemsa and observed under a light microscope. The parasite burden was determined by the following infection index: mean number of infected macrophages x mean number of amastigotes per macrophage / 100 macrophages. At least 200 macrophages were counted per tested concentration.

Cytotoxicity on Mammalian Cells

NCTC cells-clone L929 ($6x10^4$ cells/well) were seeded and incubated with test samples (maximal concentration of 150 µM)) for 48 h at 37 ºC in a 5% CO_2 incubator and the 50% cytotoxic concentration (CC_{50}) was determined by the MTT assay, as above described. For all assays, the optical density was

determined in a plate spectrophotometer reader (FilterMax F5, Molecular Devices, USA) at 570 nm. Mammalian cells were incubated without test samples and used as the viability control (100% viability).

Reference

1. Martins, L. F; Mesquita, J. T.; Pinto, E. G.; Costa-Silva, T. A.; Borborema, S. E.; Galisteo Junior, A. J.; Neves, B. J.; Andrade, C. H.; Shuhaib, Z. A.; Bennett, E. L.; Black, G. P.; Harper, P. M.; Evans, D. M.; Fituri, H. S.; Leyland, J. P.; Martin, C.; Roberts, T. D.; Thornhill, A. J.; Vale, S. A.; Howard-Jones, A.; Thomas, D. A.; Williams, H. L.; Overman, L. E.; Berlinck, R. G. S.; Murphy, P. J.; Tempone, A. G. *J. Nat. Prod*. **2016**, *79*, 2202–2210.

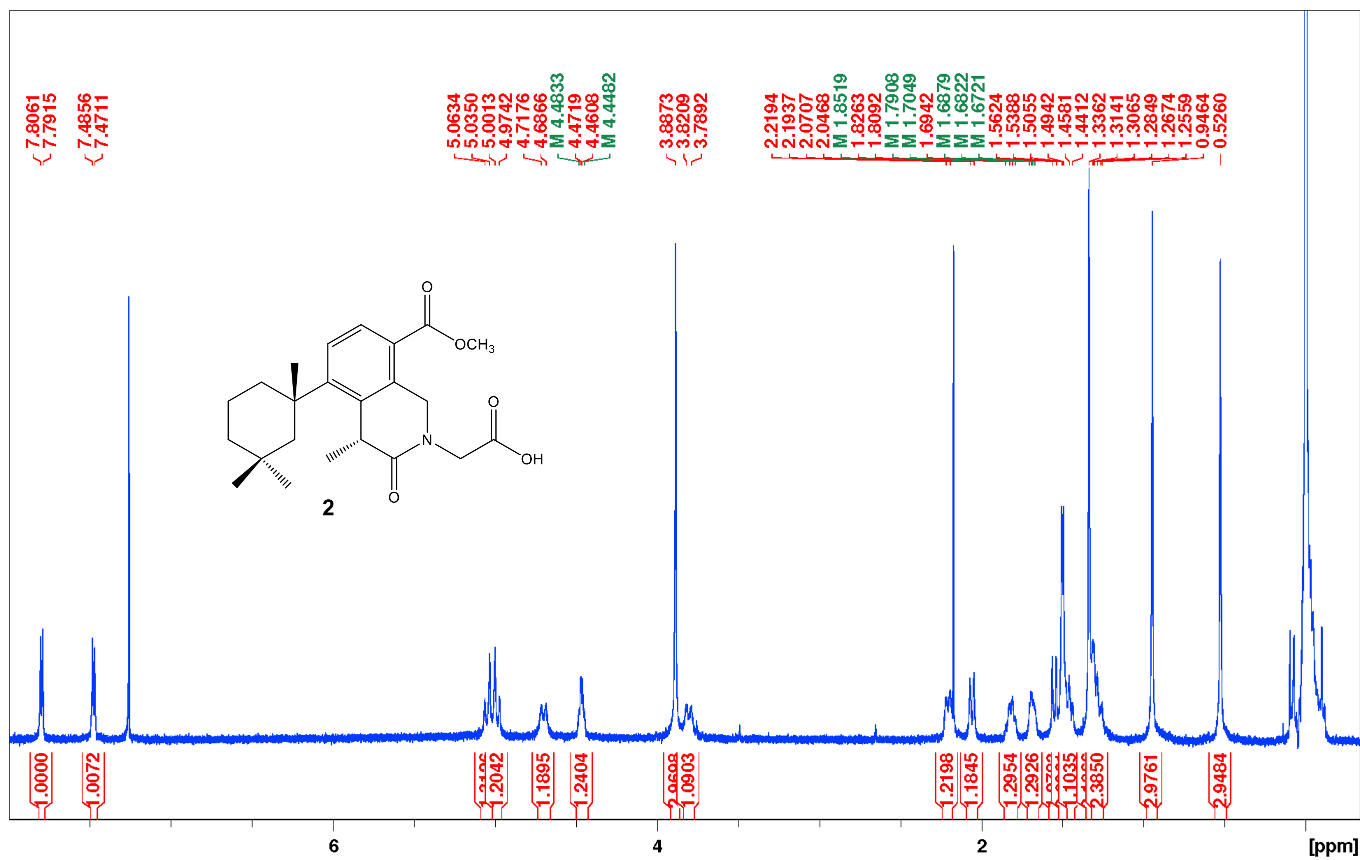

Figure S2. ^{1}H NMR Spectrum of the Oxeatine (**2**) in $CDCl_3$ at 600 MHz.

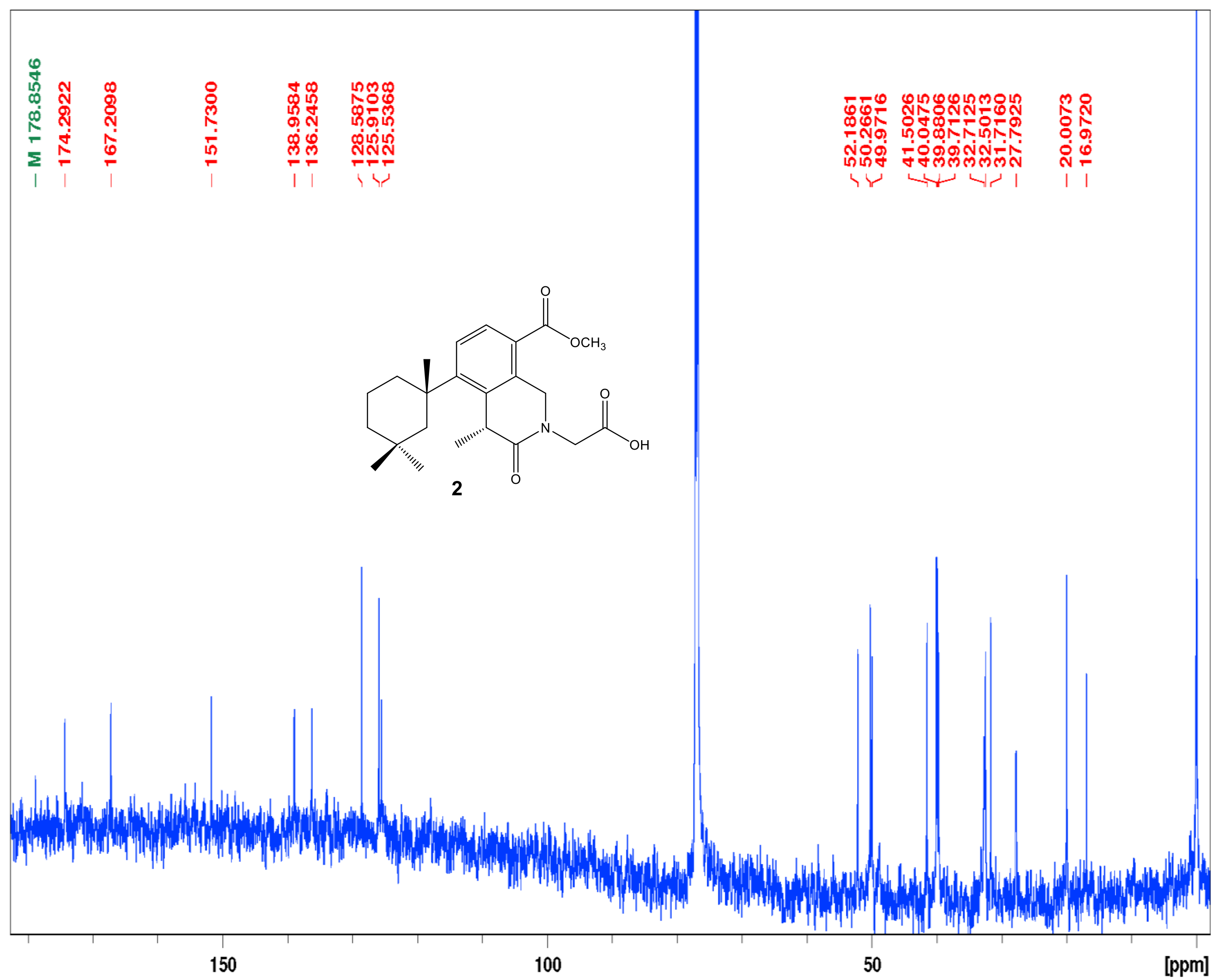

Figure S3. ^{13}C NMR Spectrum of oxeatine (**2**) in $CDCl_3$ at 150 MHz.

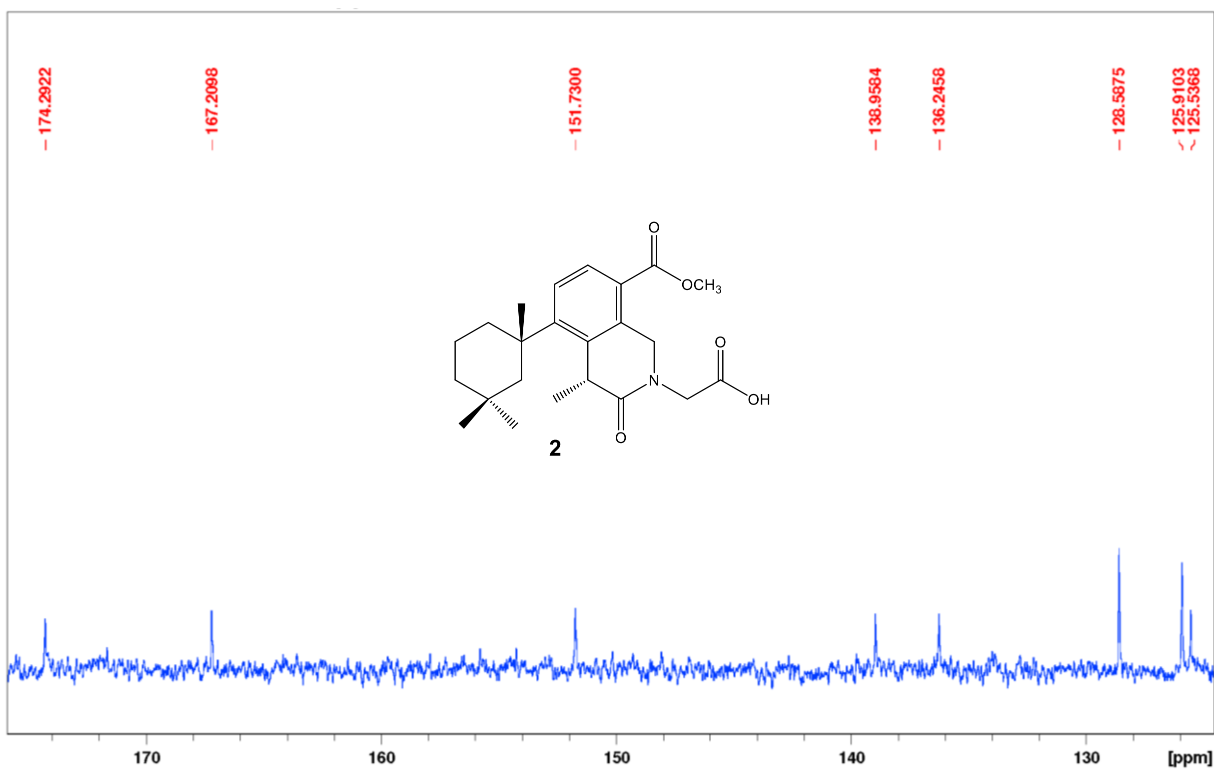

Figure S4. Expansion of the ^{13}C NMR Spectrum of oxeatine (**2**) in $CDCl_3$ at 150 MHz.

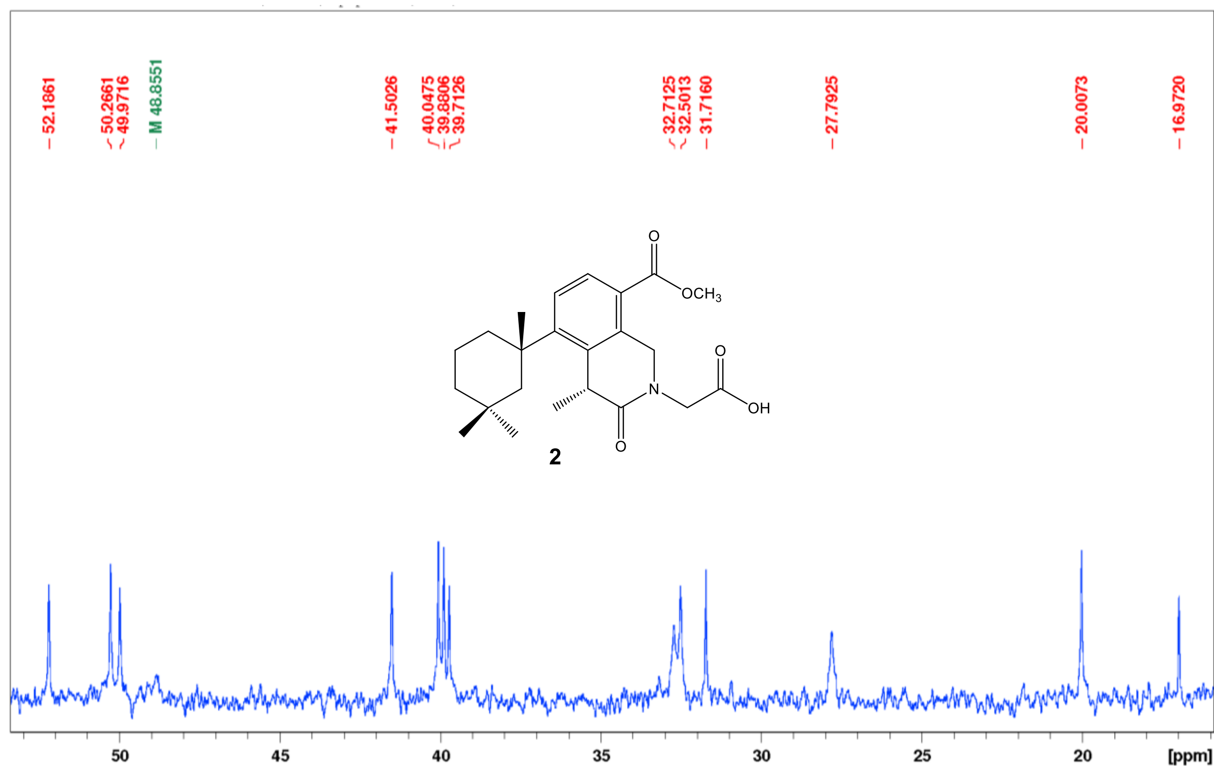

Figure S5. Expansion of the ^{13}C NMR Spectrum of oxeatine (**2**) in $CDCl_3$ at 150 MHz.

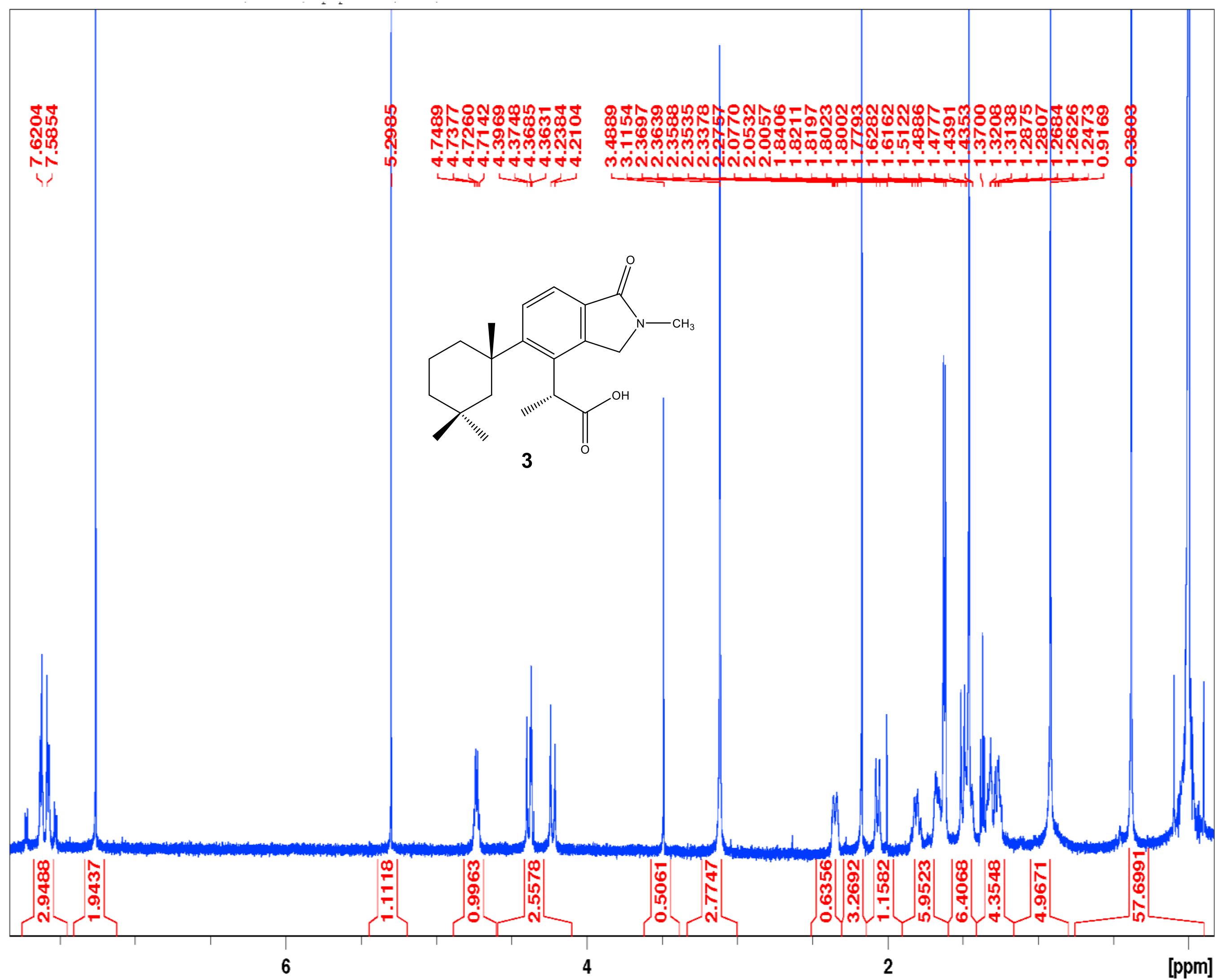

Figure S6. ^{1}H NMR Spectrum of oxeatamide H (**3**) in $CDCl_3$ at 600 MHz.

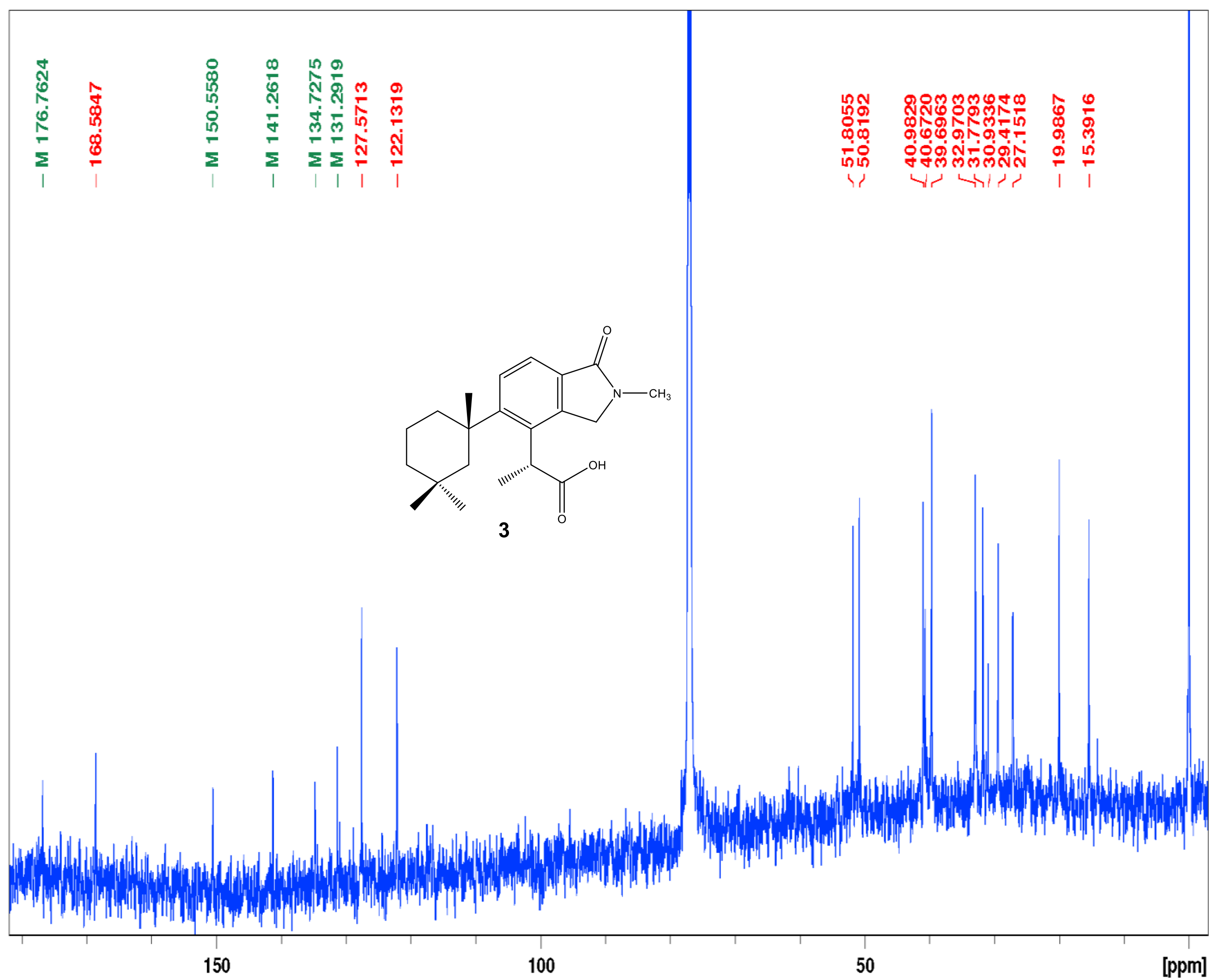

Figure S7. ^{13}C NMR Spectrum of oxeatamide H (**3**) in $CDCl_3$ at 150 MHz.

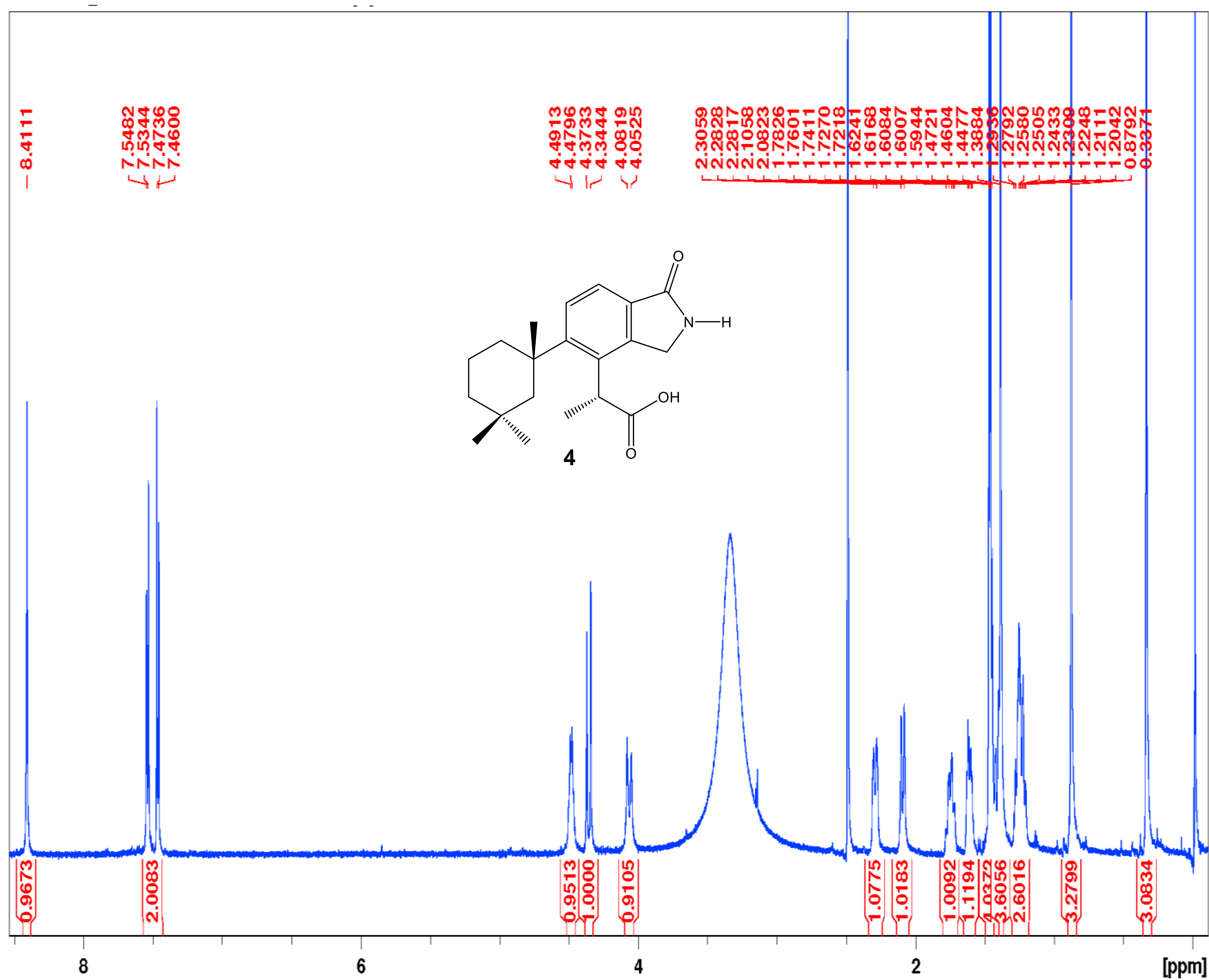

Figure S8. ¹H NMR Spectrum of oxeatamide I (**4**) in DMSO-d_6 at 600 MHz.

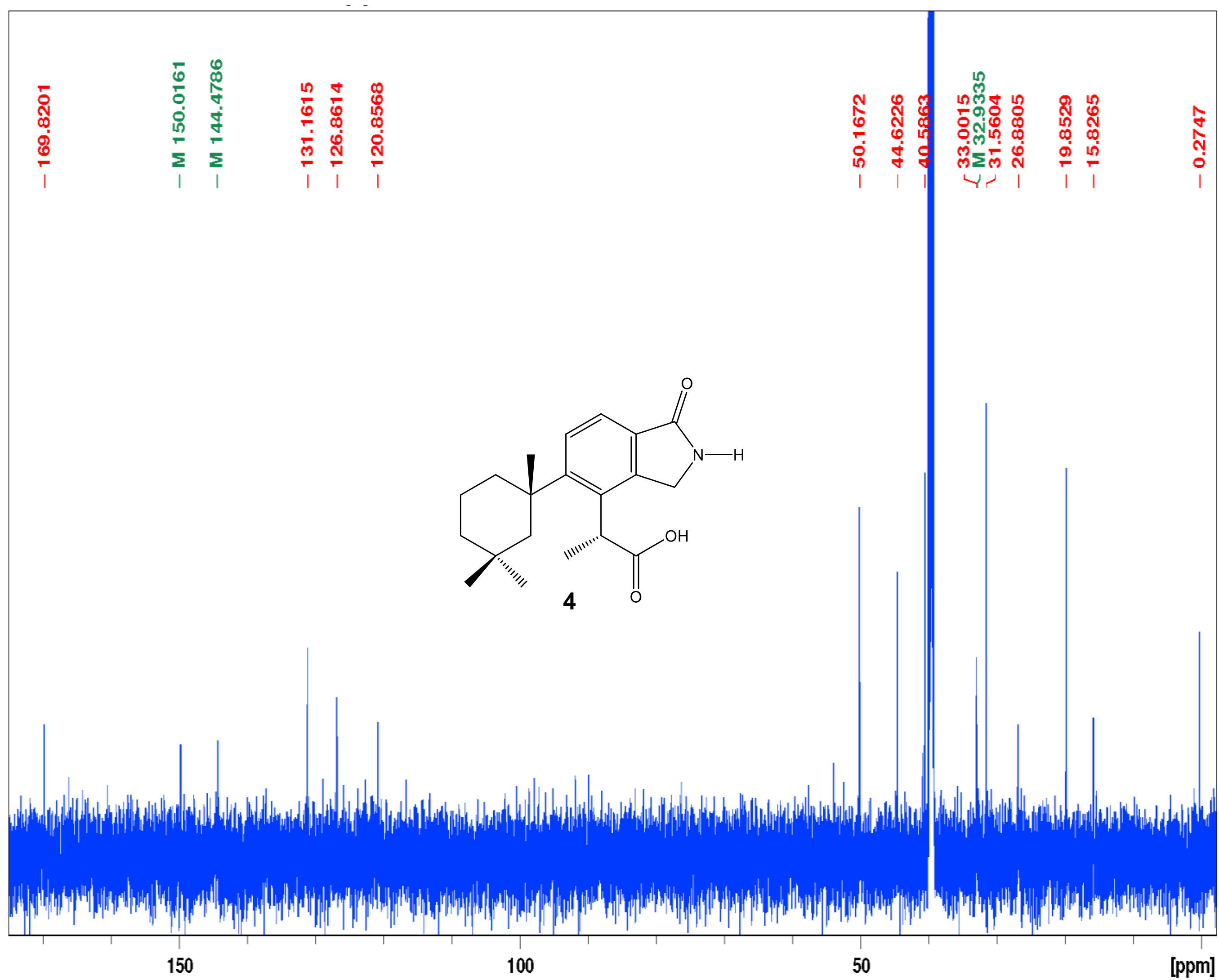

Figure S9. ^{13}C NMR Spectrum of the oxeatamide I (**4**) in DMSO-d_6 at 150 MHz.

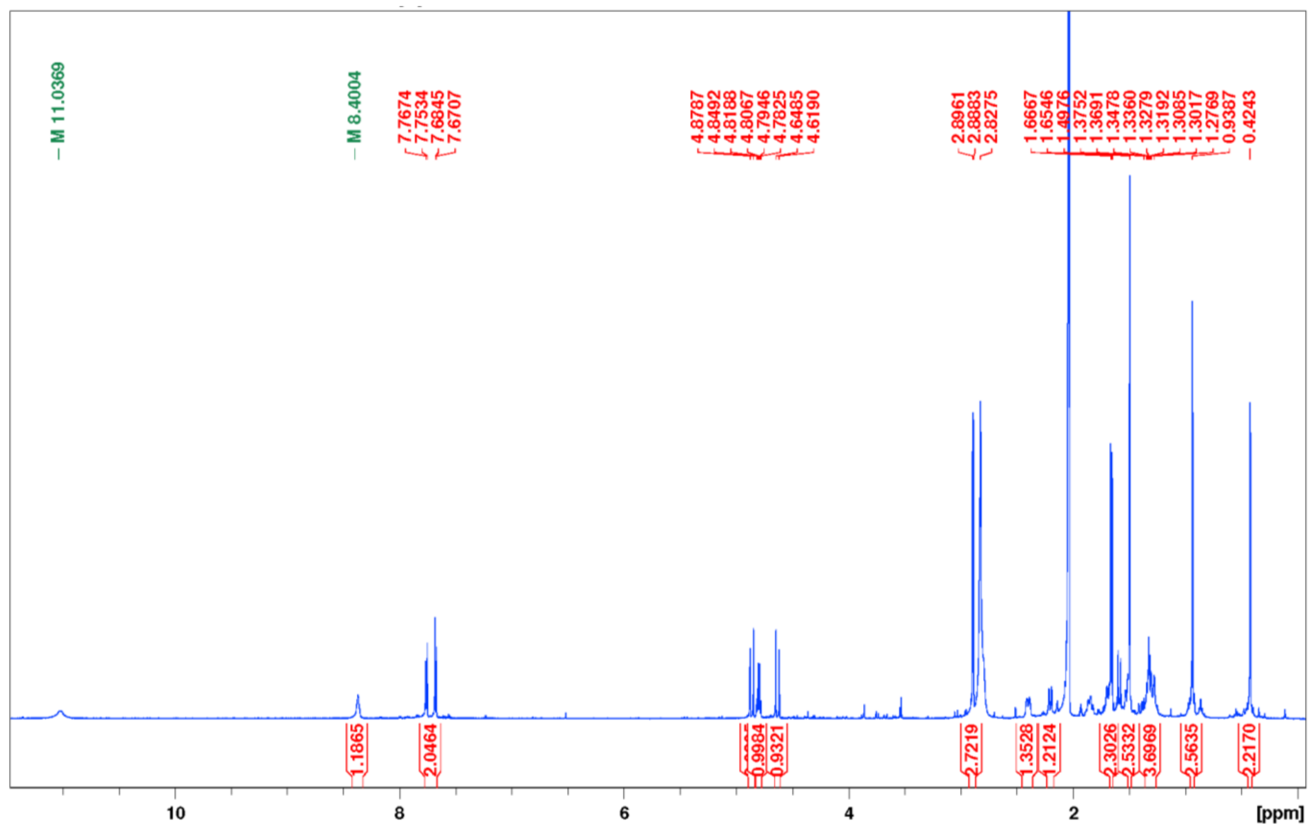

Figure S10. ^{1}H NMR Spectrum of the oxeatamide J (**5**) in acetone-d_6 at 600 MHz.

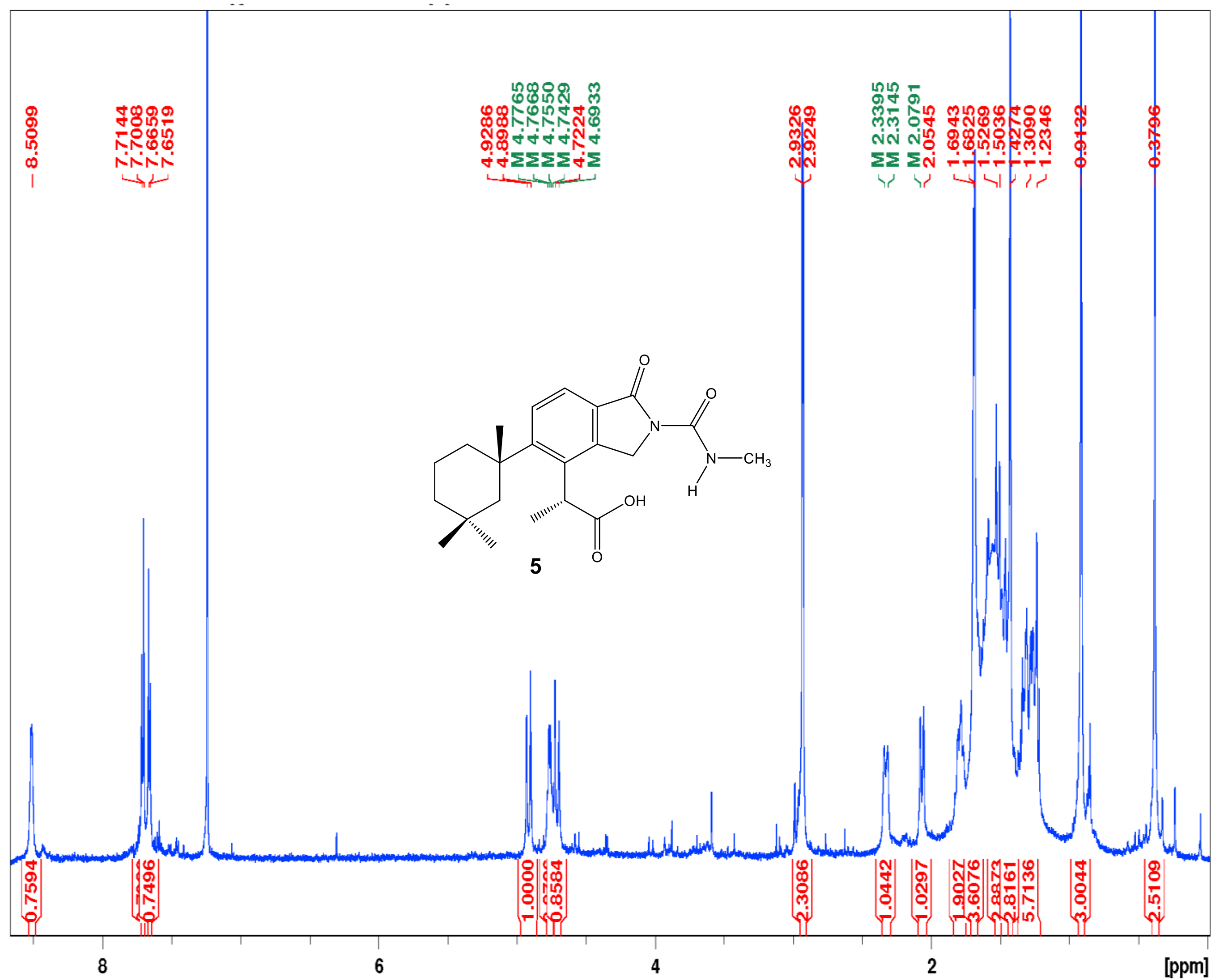

Figure S11. ^{1}H NMR Spectrum of the oxeatamide J (**5**) in DMSO-d_6 at 600 MHz.

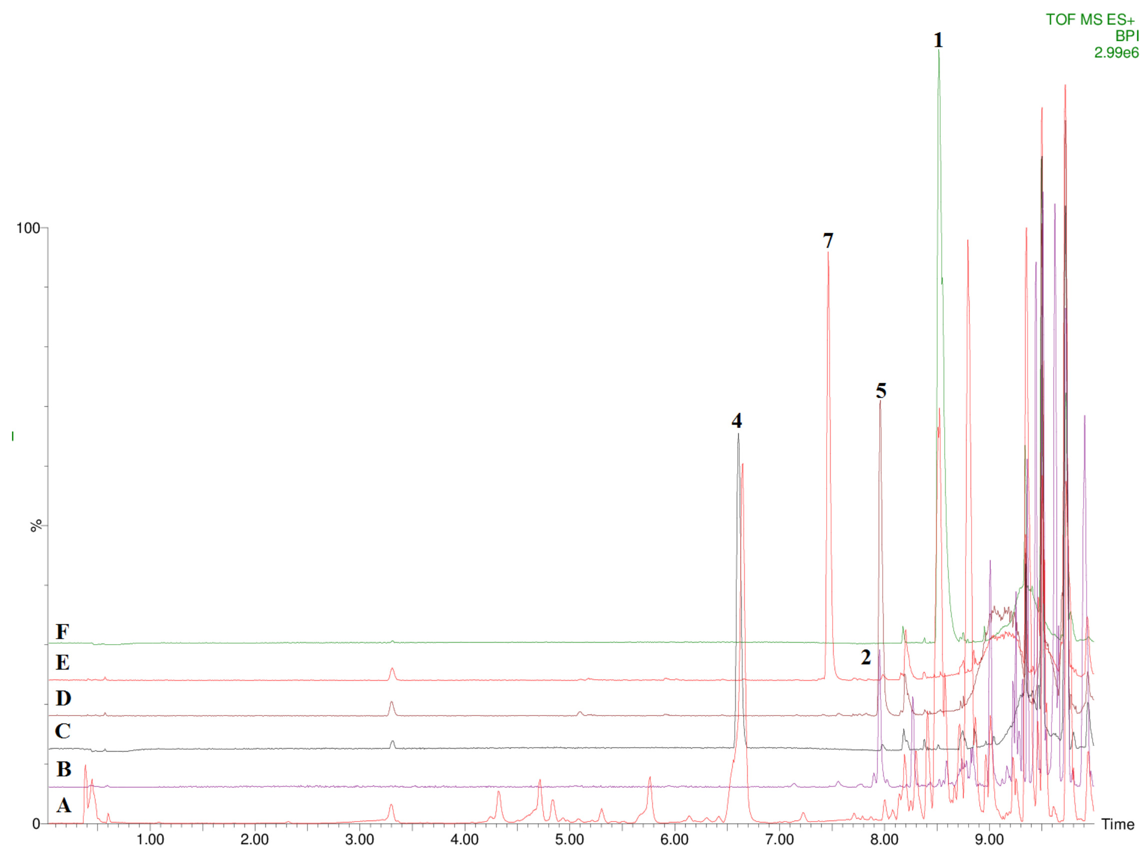

Figure S12. UPLC-QTOF trace of the extract of the mantle of *F. grahami* with rearranged diterpenes isolated from *Darwinella* cf. *oxeata*. Line A: mantle extract of *F. grahami*. Line B: Membranolide (**1**). Line C: Oxeatamide A methyl ester (**7**). Line D: Oxeatamide J (**5**). Line E: Oxeatamide I (**4**). Line F: Oxeatine (**2**). Conditions: Acquity UPLC BEH C_{18} (1.7 µm, 2.1 × 100 mm) column; eluent: gradient of MeCN (0.01% HCO_2H) in H_2O (0.01% HCO_2H), starting at 10% to 98% MeCN (0.01% HCO_2H) in 8 min. Detection: MSE continuum during 2 min, *m/z* 185−1.000 molecular weight range; detection mode: ESI(+); scan time: 0.2 s; ramp collision energy: 20−30 V.