Supporting Information

Isolation of bioactive compounds from sunflower leaves (*Helianthus annuus* L.) extracted with Supercritical Carbon Dioxide

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Fractionation and Isolation.

Fraction B (hexane/ethyl acetate 90:10 v/v, 343 mg), was subjected to column chromatography (250 mm × 40 mm i.d., 175 g of silica gel 60 A) and eluted with hexane/acetone mixtures of increasing polarity from 20 to 100% in acetone, increasing by 10% each time and finishing with 100% methanol (250 mL of each polarity) to afford 4 fractions B1-B4.

Fraction B1 (18.6 mg, 5 mg/injection) was purified by HPLC (Luna 10 µ, silica, semipreparative column) using hexane/acetone (90:10 v/v, flow 3 mL/min) with IR detection to yield **1** (6.7 mg, t_R 6.7 min). Fraction B2 (180.5 mg) was subjected to column chromatography (200 mm × 40 mm i.d., 80 g of silica gel 60 A) using hexane/acetone mixtures of increasing polarity from 30 to 100% in acetone increasing by 10% each time and finishing with 100% methanol (100 mL of each polarity) to afford 3 fractions (B2.1, B2.2, B2.3). Fraction B2.2 (90 mg, 5 mg/injection) was purified by HPLC (Luna 10 µ, silica, semipreparative column, IR detection) using hexane/acetone (90:10 v/v, flow 3 mL/min) to yield **1** (1.7 mg, t_R 6.9 min) along with a second component (45 mg, t_R 12 min). This material was further purified by HPLC using hexane/acetone (85:15 v/v, flow 3 mL/min, 5 mg/injection, Luna 10 µ, silica, semipreparative column, IR detection) to yield **3** (11.3 mg, t_R 3.2 min) and **6** (7.8 mg, t_R 4.1 min). Fraction B2.3 (39.4 mg, 5 mg/injection) was purified by HPLC (Luna 10 µ, silica, semipreparative column) using hexane/acetone 85:15 v/v (flow 3 mL/min) to yield two components: **4** (5.3 mg, t_R 5.9 min) and **7** (7.9 mg, t_R 6.7 min). Fraction C (hexane/ethyl acetate 80:20 v/v, 1235 mg) was subjected to column chromatography (200 mm × 60 mm i.d., 1200 g of silica gel 60 A) using hexane/acetone mixtures of increasing polarity from 20 to 100% in acetone, increasing by 10% each time and finishing with 100% methanol (500 mL of each polarity) to afford 7 fractions C1–C7. Fraction C1 (320 mg) after TLC eluted with hexane/acetone (80:20 v/v), was purified

by HPLC (Luna 10 µ, silica, semipreparative column, IR detection) using hexane/acetone (85:15 v/v, flow 3 mL/min, 5 mg/injection) to yield three components: **2** (27.8 mg, t_R 6.9 min), **5** (4.8 mg, t_R 7.6 min), and an isomeric mixture of **16** and **17** (8.25 mg, t_R 8.9 min). Fraction C2 (200.2 mg) was subjected to column chromatography (200 mm x 30 mm i.d., 60 g of silica gel 60 A) using hexane/acetone mixtures of increasing polarity from 20 to 100% in acetone increasing by 10% each time and finishing with 100% methanol (150 mL of each polarity), to afford two fractions: C2.1 and C2.2. Fraction C2.1 (80 mg) was purified by HPLC (Luna 10 µ, silica, semipreparative column, 5 mg/injection, IR detection) using hexane/acetone (90:10 v/v, with 1% acetic acid, flow 3 mL/min) to yield **5** (3.3 mg, t_R 9.6 min) and **18** (3.1 mg, t_R 13 min),

Fraction C2.2 (53.2 mg, 5 mg/injection) was purified using the same conditions as for fraction C2.1, but with hexane/acetone 85:15 v/v as the eluent, to yield **10** (10.7 mg, t_R 4.7 min), and **15** (3.9 mg, t_R 10 min). Fraction C3 (171 mg) was purified by HPLC (Luna 10 µ, silica, semipreparative column, using hexane/ethyl acetate 70:30 v/v, flow 3 mL/min, 5 mg/injection, IR detection) and two peaks were obtained for impure compounds that were later purified by analytical column chromatography (Luna 5 µ, silica, hexane/ethyl acetate 80:20 v/v, flow 1 mL/min, 1 mg/injection, IR detection). The purified compounds were **7** (3.7 mg, t_R 7.1 min), and **18** (5.7 mg, t_R 9.9 min). Fraction D (hexane/ethyl acetate 70:30 v/v, 349.6 mg) was subjected to column chromatography (200 mm × 40 mm i.d., 140 g of silica gel 60 A) using hexane/acetone mixtures of increasing polarity from 20 to 100% in acetone, increasing by 10% each time and finishing with 100% methanol (200 mL of each polarity). A total of 4 fractions were obtained according to their chromatographic similarity (D1–D4). Fraction D1 (61.4 mg, 5 mg/injection) was purified by HPLC (Luna 10 µ, silica, semipreparative

column, using hexane/ethyl acetate 80:20 v/v, flow 3 mL/min, IR detection) to afford 3 compounds: **4** (3.7 mg, t_R 5 min), **5** (4.3 mg, t_R 6.3 min) and **9** (7.7 mg, t_R 7.2 min). Fraction D3 (122.6 mg, 5 mg/injection) was purified by HPLC (Luna 10 µ, silica, semipreparative column, using hexane/ethyl acetate 85:15 v/v, flow 3 mL/min, IR detection) to yield 4 compounds: **8** (9.5 mg, t_R 6.4 min), **11** (7.8 mg, t_R 10 min), and **19** and **20** as an isomeric mixture (10.4 mg, t_R 12.3 min). Fraction E (hexane/ethyl acetate 50:50 v/v, 2.5 g) was subjected to column chromatography (210 mm × 70 mm i.d., 1300 g of silica gel 60A) using chloroform/ethyl acetate mixtures of increasing polarity from 0 to 100% in ethyl acetate, increasing by 20% each time and finishing with 100% methanol (500 mL of each polarity), to afford 6 fractions (E1–E6). Fraction E2 (1.5 g) was mainly a sub-fraction of fraction E and this was subjected to column chromatography (210 mm × 60 mm i.d., 1250 g of silica gel 60 A) using hexane/acetone mixtures of increasing polarity from 20 to 100% in acetone increasing by 20% each time and finishing with 100% methanol (500 mL of each polarity), to afford 5 sub-fractions (E2.1–E2.5). Fraction E2.1 (98.3 mg) after successive purifications by HPLC (Luna 10 µ, silica, semipreparative column, using first hexane/ethyl acetate 80:20 and then hexane/ethyl acetate 75:25 v/v, flow 3 mL/min, 5 mg/injection, IR detection), yielded four compounds: **8** (3.1 mg, t_R 4.5 min), **50** (3.1 mg, t_R 10.5 min), **22** (9.3 mg, t_R 12.3 min), and **21** (8.1 mg, t_R 13.1 min). E2.2 (550.5 mg) was subjected to column chromatography (250 mm x 40 mm i.d., 175 g of silica gel 60 A) using hexane/acetone mixtures of increasing polarity from 20 to 100% in acetone, increasing by 20% each time and finishing with 100% methanol (250 mL of each polarity), to afford 3 sub-fractions (E2.2.1–E2.2.3) and, after successive purifications by HPLC for both sub-fractions (Luna 10 µ, silica, semipreparative column, hexane/ethyl acetate 70:30 v/v, flow 3 mL/min, 5 mg/injection, IR detection), the following compounds were obtained:

30 (21.7 mg, t_R 7.1 min), **9** (3.0 mg, t_R 7.5 min), **51** (2.8 mg, t_R 11 min), **22** (2.9 mg, t_R 11.8 min), **12** (8.8 mg, t_R 12 min), **41** (9.7 mg, t_R 13 min), **33** (5.3 mg, t_R 14.2 min), **32** (4.7 mg, t_R 15.1 min), **34** (6.1 mg, t_R 15.9 min), **36** (3.2 mg, t_R 16.9 min), **43** (5.8 mg, t_R 17.3 min), **42** (4.5 mg, t_R 17.9 min), **45** (3.6 mg, t_R 18.4 min), **39** (5.9 mg, t_R 19.6 min) and **40** (8.6 mg, t_R 20.6 min). Fraction E2.3 (272.2 mg, 5 mg/injection) was purified by HPLC (Luna 10 µ, silica, semipreparative column, using hexane/ethyl acetate 75:25 v/v, flow 3 mL/min, IR detection) to afford 8 components that, after several successive injections under the same conditions on an analytical column (Luna 5 µ, silica, using hexane/ethyl acetate 70:30 v/v, flow 1 mL/min, 1 mg/injection, IR detection) for final purification yielded the following compounds: **24** (7.9 mg, t_R 11.8 min), **25** (6.8 mg, t_R 12 min), **23** (7.1 mg, t_R 12.3 min), **26** (8.7 mg, t_R 14.3 min), **27** (3.8 mg, t_R 15.3 min), **45** (14.1 mg, t_R 18.1 min), **46** (3.3 mg, t_R 19.9 min), and **28** (8.3 mg, t_R 21.3 min). Fraction E3 (589.7 mg) was subjected to column chromatography (250 mm × 40 mm i.d., 175 g of silica gel 60 A) using hexane/acetone mixtures of increasing polarity from 20 to 100% in acetone, increasing by 20% each time and finishing with 100% methanol (250 mL of each polarity), to afford 3 sub-fractions (E3.1–E3.3) and, after successive purifications by HPLC (Luna 10 µ, silica, semipreparative column, hexane/ethyl acetate 60:40 v/v, flow 3 mL/min, 5 mg/injection, IR detection), and subsequently on an analytical column (Luna 5 µ, silica, hexane/ethyl acetate 60:40 v/v, flow 1 mL/min, 1 mg/injection, IR detection), the following six compounds were obtained: **13** (4.3, t_R 7.2 min), **14** (7.4 mg, t_R 8 min), **37** (2.4 mg, t_R 12.3 min), **29** (6.8 mg, t_R 15.3 min), **31** (3.8 mg, t_R 16.3 min) and **38** (3.1 mg, t_R 17.5 min). Fraction F (hexane/ethyl acetate 20:80 v/v, 644.3 mg) was subjected to column chromatography (250 mm × 40 mm i.d., 175 g of silica gel 60A) using chloroform/ethyl acetate mixtures of increasing polarity from 0 to 100% in ethyl acetate, increasing by 20% each time and finishing with 100%

methanol (250 mL of each polarity), to afford 4 fractions F1–F4. Fraction F1 (88 mg) was purified by HPLC (Luna 10 μ, silica, semipreparative column, using hexane/ethyl acetate 55:45 v/v, flow 3 mL/min, 5 mg/injection, IR detection) to afford 5 impure compounds which were purified using an analytical column (Luna 5 μ, silica, hexane/acetone 60:40 v/v, flow 1 mL/min, 1 mg/injection, IR detection) to yield **22** (4.2, t_R 7.1 min), **52** (3.4 mg, t_R 8.6 min), **44** (8.3 mg, t_R 14.5 min), **49** (10.1 mg, t_R 15.3 min) and **38** (3.5 mg, t_R 16.9 min). Fraction F2 (95.3 mg) was purified by HPLC (Luna 10 μ, silica, semipreparative column, hexane/acetone 55:45 v/v, flow 3 mL/min, 5 mg/injection, IR detection) to yield four compounds, which were purified using an analytical column with the same conditions of polarity (Luna 5 μ, silica, hexane/acetone 55:45 v/v, flow 1 mL/min, 1 mg/injection, IR detection), to give **44** (3.2 mg, t_R 10.5 min), **48** (8.8 mg, t_R 11.2 min), **35** (6.8 mg, t_R 12 min) and **47** (9.8 mg, t_R 13.2 min).

Identified compounds

The isolated compounds were identified from their spectroscopic data (^{1}H NMR, ^{13}C NMR, HMQC, COSY, HMBC, IR, and MS) and by comparison with the data reported in the literature for linoleic acid **1**,[1] linolenic acid **2**,[2] (–)-kaur-16-en-19-oic acid **3**,[3] grandifloric acid **4**,[3] angeloylgrandifloric acid **5**,[3] *ent*-15*β*,16*β*-epoxykauran-19-oic acid **6**,[4] (–)- 15*β*,16*β*-epoxy-17-hydroxy-kauran-19-oic acid **7**,[5] ent-17-oxo-kaur-15(16)-en-19-oic acid **8**,[5] nor-ent-kauranone acid **9**,[6] (–)-17-hydroxy-ent-isokaur-15-en-oic acid **10**,[5] (–)-17-hydroxy-16*β*-kauran-19-oic acid **11**,[6] (–)-17-hydroxy-16*α*-kauran-19-oic acid **12**,[6] 16*α*-hydroxy (–)-kauran-19-oic acid **13**,[7] 16*β*-hydroxy (–)-kauran-18-oic acid **14**,[8] 7,11,15-trimethyl-3-methylenehexadecan-1,2-diol **15**,[9] *α*-amyrin **16**,[10] *β*-amyrin **17**,[10] stigmasterol **18**,[11] 5*α*,6*α*-epoxystigmasterol **19**,[12] 5*β*,6*β*-epoxystigmasterol **20**,[12] esculetin **21**,[13] tambulin **22**,[14] gardenin B **23**,[15] pectolinarigenin **24**,[15] hymenoxin **25**,[16] heliannone A **26**,[17] kukulkanin B **27**,[17] heliannone B **28**,[17] heliannone C **29**,[17] heliannuol A **30**,[18] 6-hydroxy-megastigm-7-en-3,9-dione **32**,[19, 20] 6-hydroxy-megastigm-4,7-dien-3,9-dione **33**,[21] annuionone D **34**,[22] boscialin **35**,[23] angeloylcumambrin B **39**,[24] annuolide G **40**,[25] 8-angelyloxy-2-oxo-guaia-1(10),3,11(13)-trien-6,12-olide **41**,[26] annuolide C **42**,[27] helivypolide D **43**,[28] niveusine B **44**,[29] leptocarpin **45**,[30, 31] heliangin **46**,[32] 15-hydroxy-3-dehydrodeoxytifruticin **47**,[33] 1*β*,2*α*-epoxytagitinin C **48**,[34] helivypolide G **49**,[35] eudesm-4(15)-en-1β,6α-diol **50**,[36] loliolide **51**,[37] and pinorresinol **52**.[38]

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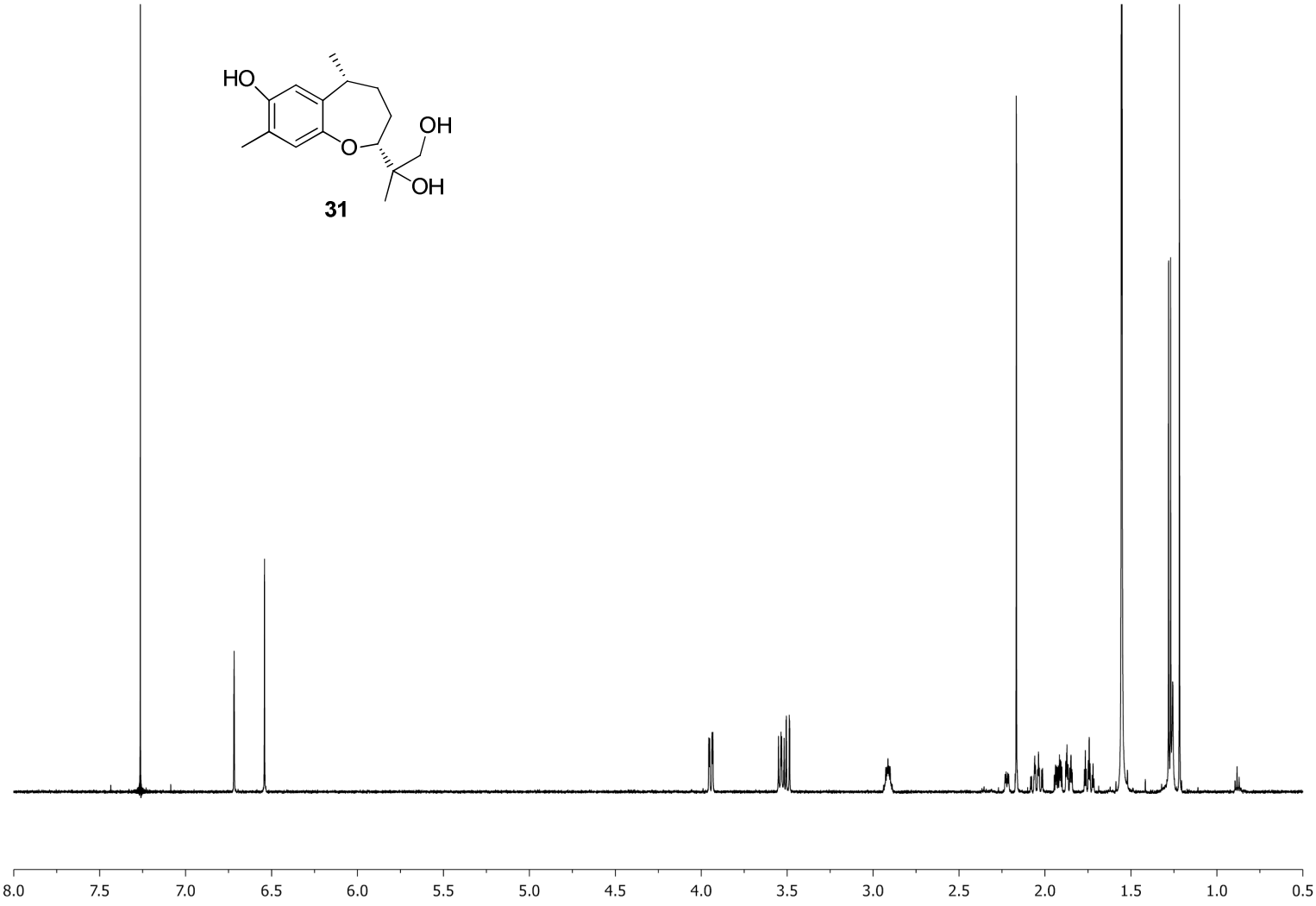

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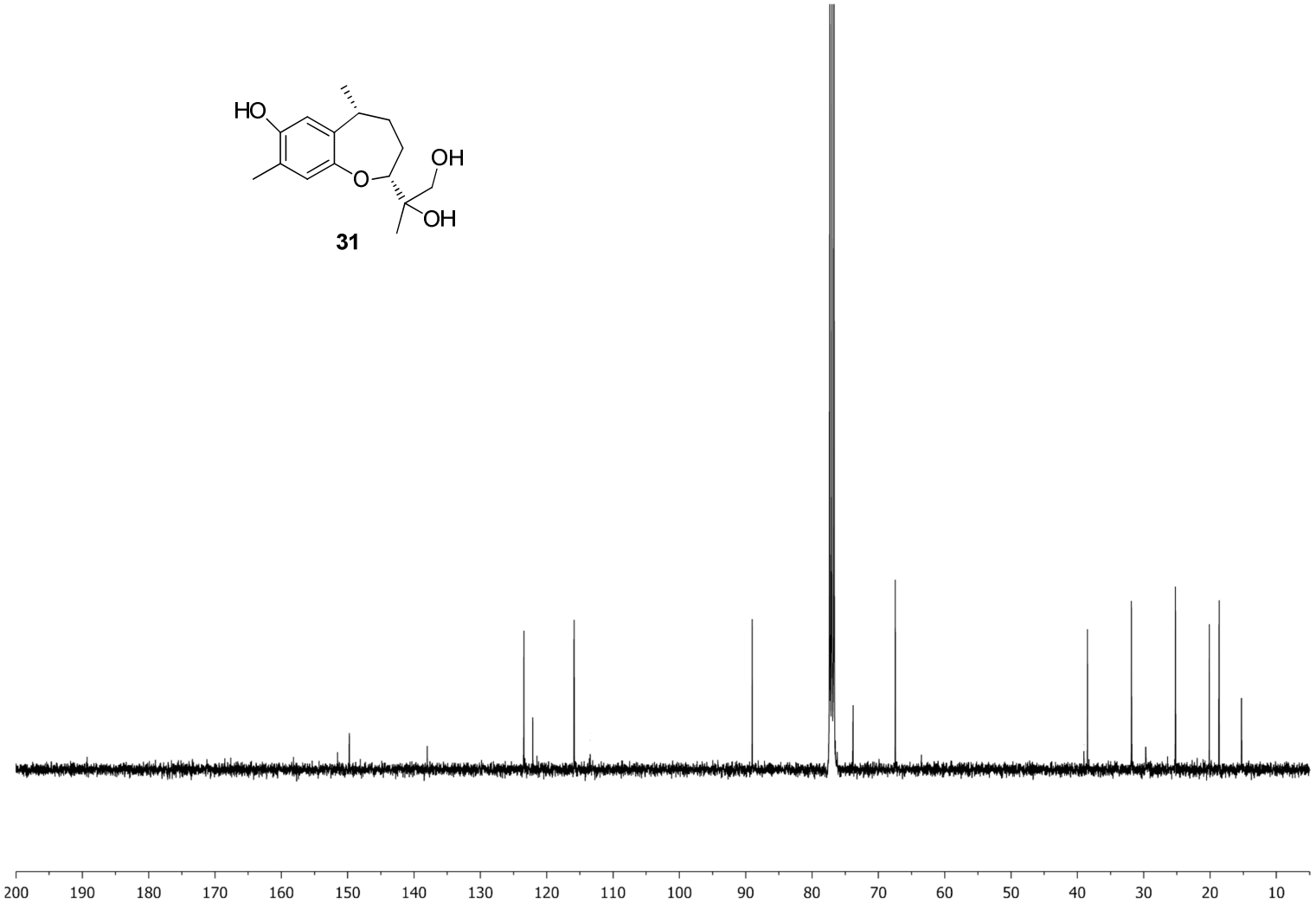

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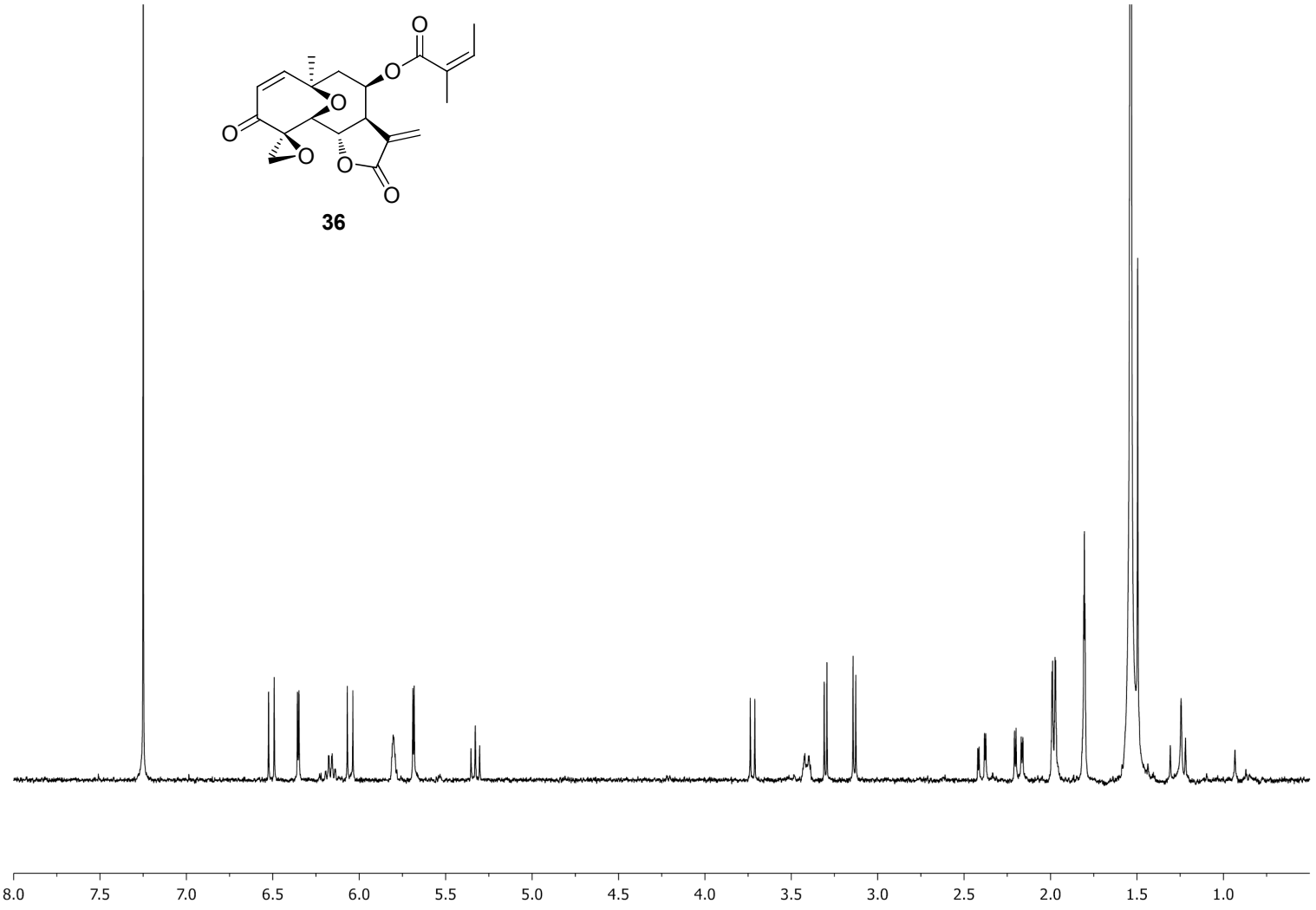

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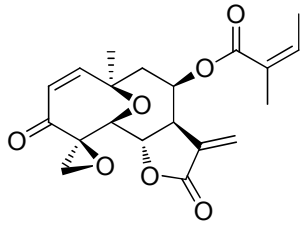

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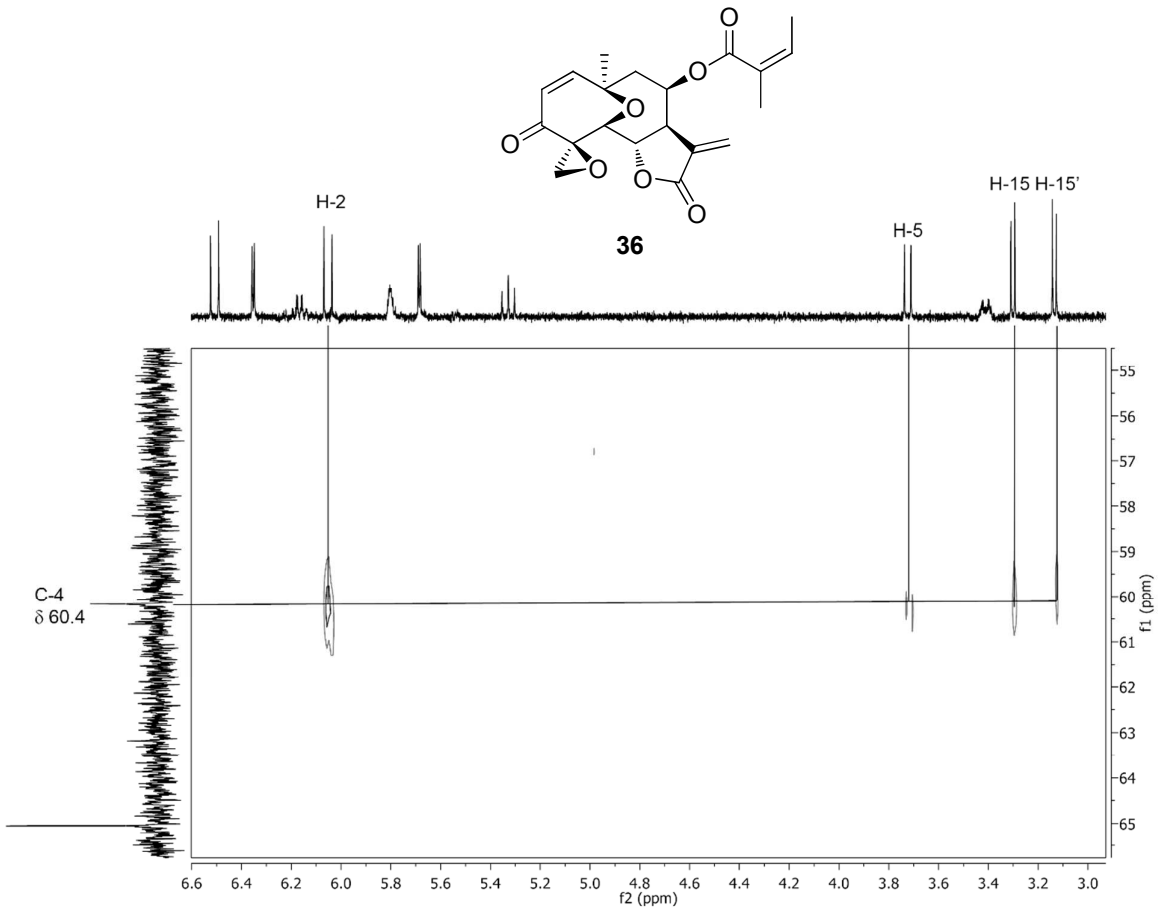

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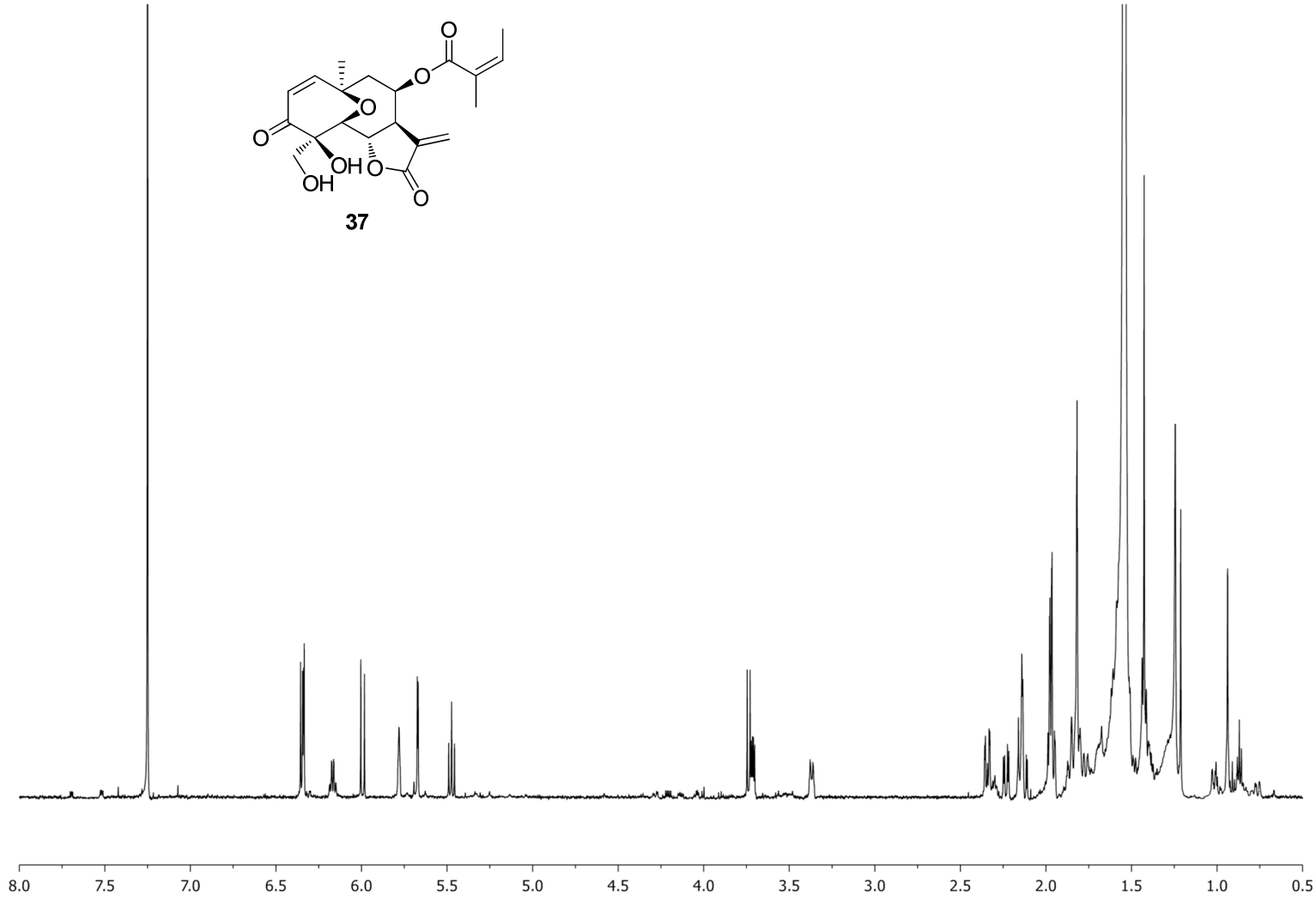
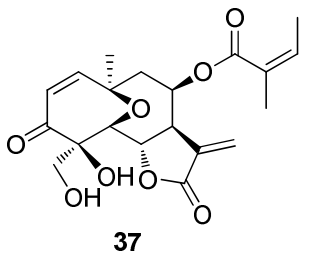
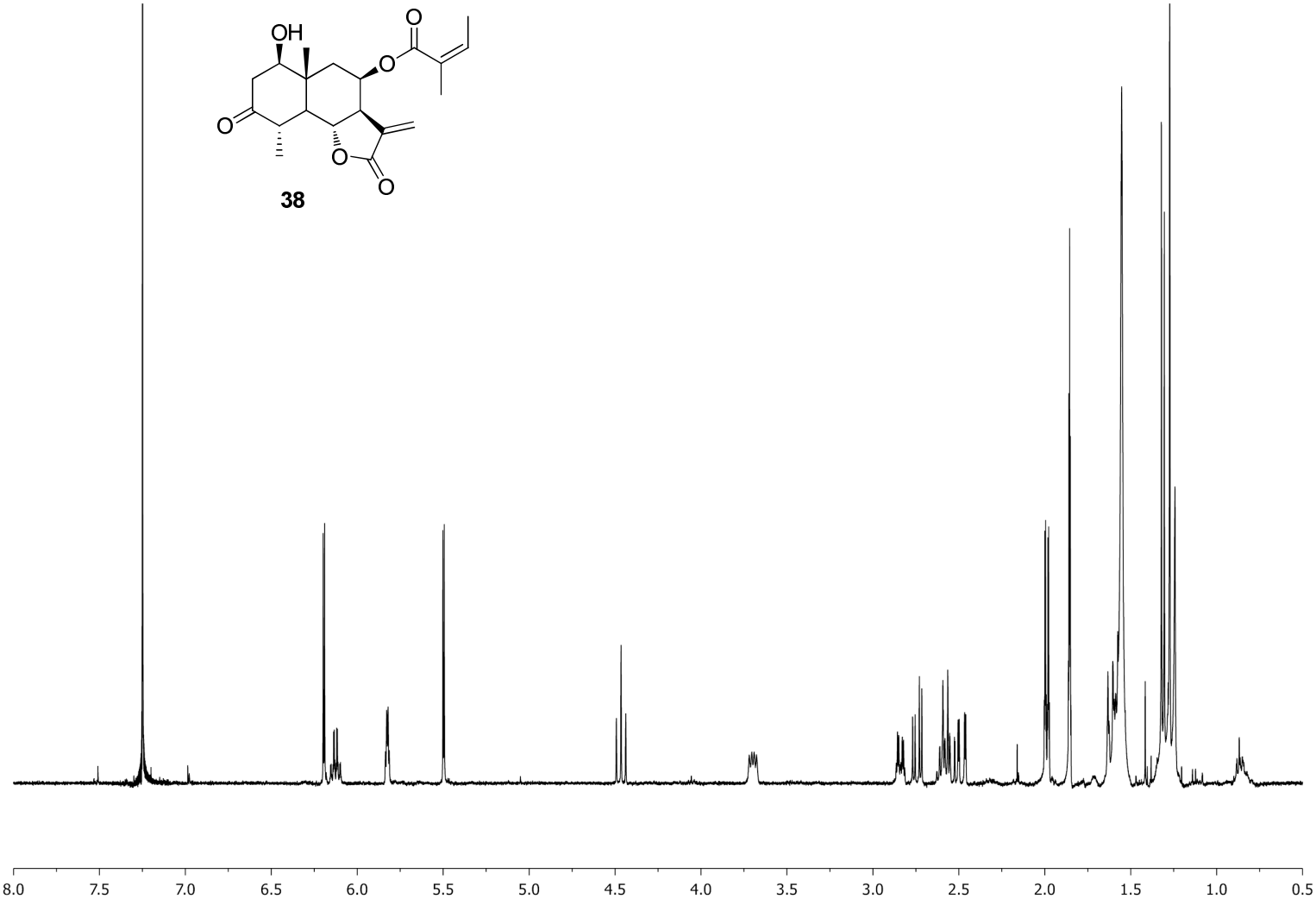


^{1}H NMR spectrum of helivypolide L (37) (600 MHz, $CDCl_3$).

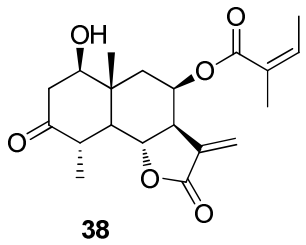


37

^{13}C NMR spectrum of helivypolide L (37) (125 MHz, $CDCl_3$).

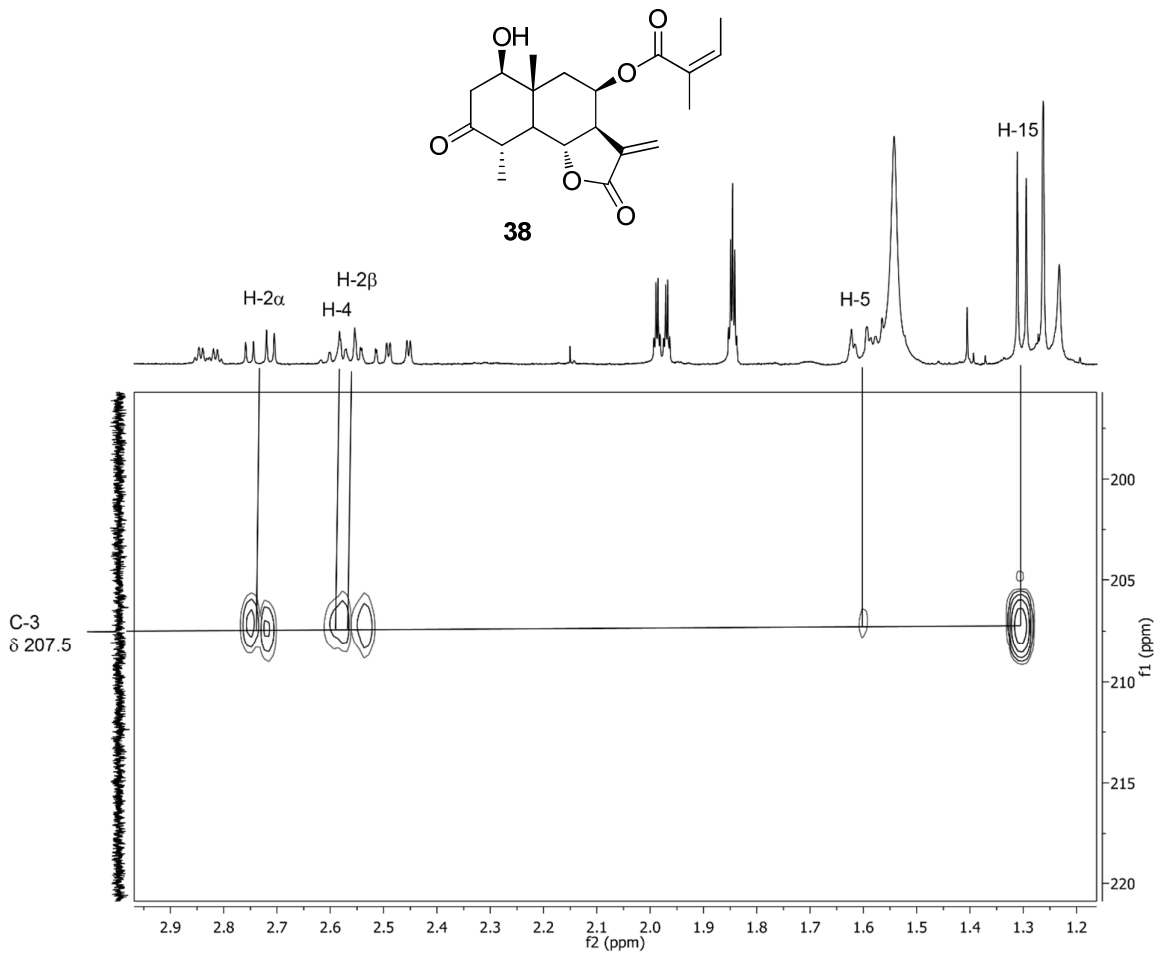


^{1}H NMR spectrum of helieudesmanolide B (38) (600 MHz, $CDCl_3$).



38

^{13}C NMR spectrum of helieudesmanolide B (38) (125 MHz, $CDCl_3$).



Enlargement g-HMBC spectrum of helieudesmanolide B (38) (600 MHz, $CDCl_3$).