Supporting Information

Cerebrosides of the Halotolerant Fungus *Alternaria raphani* Isolated from a Sea Salt Field

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List of Supporting Information

Bioassay Protocols

Cytotoxic Assays. In the MTT assay, cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Cell suspensions, 200 μL, at a density of 5×10^4 cell mL^{-1} were plated in 96-well microtiter plates and incubated for 24 h. Then, 2 μL of the test solutions (in MeOH) were added to each well and further incubated for 72 h. The MTT solution (20 μL, 5 mg/mL in IPMI-1640 medium) was then added to each well and incubated for 4 h. Old medium containing MTT (150 μL) was then gently replaced by DMSO and pipetted to dissolve crystals formed. Absorbance was then determined on a Spectra Max Plus plate reader at 540 nm. In the SRB assay, 200 μL of the cell suspensions were plated in 96-well plates at a density of 2×10^5 cell mL^{-1}. Then 2 μL of the test solutions (in MeOH) was added to each well and the culture was further incubated for 24 h. The cells were fixed with 12% trichloroacetic acid and the cell layer stained with 0.4% SRB. The absorbance of the SRB solution was measured at 515 nm. The new isolates **1**–**4** were evaluated for cytotoxicity against P388 and HL-60 cancer cells with the SRB method,[25] and the A549 and BEL-7402 cancer cells with the MTT methods.[26] Vp-16 (etoposide) was used as the positive control with the IC_{50} values of 0.050 μM, 0.042 μM, 0.63 μM, and 1.03 μM, respectively.

Antimicrobial Assays. According to agar dilution method,[27] the tested strains (*Escherichia coli*, *Bacillus subtilis*) were cultivated in LB agar plates for bacteria and in YPD agar plates for *C. albicans* at 37 °C. Ciprofloxacin lactate and fluconazol was used as the positive control for bacteria and *Candida albicans* with MIC values of 3.71 μM, 7.42 μM, and 5.10 μM, respectively. Compounds **1**–**4** and positive controls were dissolved in 5% DMSO-H_2O at different concentrations from 400 to 0.78 μg/mL by the continuous 2-fold dilution methods. A 5 μL quantity of test solution was absorbed by a paper disk (5 mm diameter) and placed on the assay plates. After 24 h incubation, zones of inhibition (mm in diameter) were recorded. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration at which no microbial growth could be observed.

Antioxidative Assays. According to DPPH assay,[28] 160 μL of reaction mixtures containing test samples and 40 μL of DPPH (Sigma) in MeOH were plated in 96-cell plates and incubated in the dark for 30 min. After the reaction, absorbance was measured at 520 nm, and percent inhibition was calculated. The antioxidant activity of each sample was expressed in terms of IC_{50} (the

concentration of sample required to scavenge 50% of DPPH free radicals) and calculated from the log-dose inhibition curve. Ascorbic acid was used as the positive control with IC_{50} 22 μM.

The 18S rRNA sequence data of *Alternaria raphani* THW-18

TTATACCGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATTTGATAATACCTTACTAC

TTGGATAACCGTGGTAATTCTAGAGCTAATACATGCTGAAAATCCCGACTTCGGAAGGGATGTGT

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ATAGCCTTGCGCTGGCGACGGTTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAAGGTATT

GGCTTACCATGGTTTCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAA

ACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAG

TGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGTACAATTTAAACCT

CTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAG

CGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAAACTTGGGCCTGGCTGGCGGGTCCG

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ACGTTAGCATGGAATAATAAAATAGGGCGTGCGTTTCTATTTTGTTGGTTTCTAGAGACGCCGCA

ATGATTAACAGGAACAGTCGGGGGCATCAGTATTCAGTTGTCAGAGGTGAAATTCTTGGATTTAC

TGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAGTGAACGAAAGTTAG

GGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCGTAAACTATGCCGACTAGGGATCGGGC

GATGTTCTTTTTCTGACTCGCTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAT

TATGGTCGCAAGGCTGAAACTTAAAGAAATTGACGGAAGGTCACCACCAGGCGTGGAGCCTGCGG

CTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGATGAAATAAGGATTGACAGATTGAGA

GCTCTTTCTTGATTTTTCAGGTGGTGGTGCATGGCCGTTCTTAGTTCGTGGGGTGACTTGTCTGC

TTAaTTgCGATAACGAGCGAGACCTTACTCTGCTAAATAGCCAGGCTAACTTTGGTTGGTCGCCG

GCTTCTTAGAGAGACTATCAACTCAAGTTGATGGAAGTTTGAGGCAATAACAGGTCTGTGATGCC

CTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACAGAGCCAACGAGTTCTTTTCCTTGTTCGA

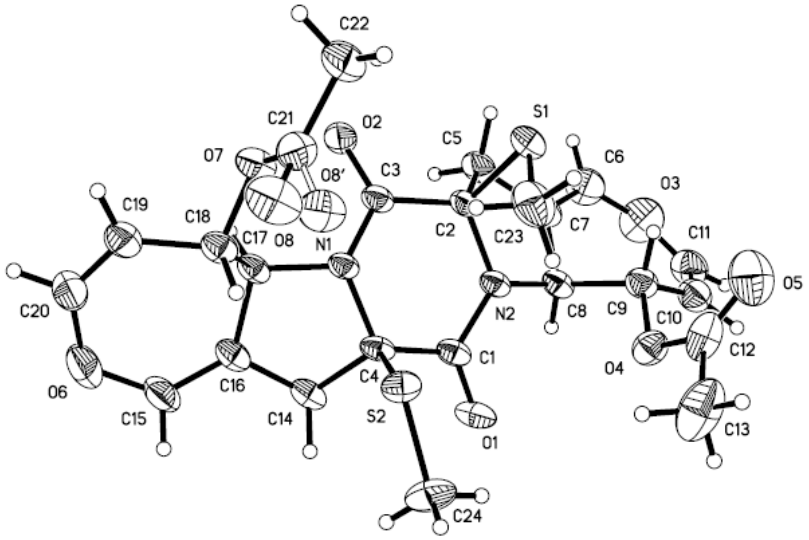
AAGAATTGGGTAATCTTGTTAAACTCTGTCGTGCTGGGGATAAAGCATTGCAATTATTGCTTTTC

AACGAGGAATGCCTAGTAAGCGCGTGTCATCAGCATGCGTTGATTACGTCCCTGACCTTTGTACA

CACCGCCCGTCGCTACTACCGATTGAATGGCTCAGTGAGGCCTTCGGACTGGCTCGAGGAGGTTG

GCAACGACCACCTCAAGCCGGAAAGTTCGTCAAACTCGG

Figure S1. X-ray crystal structure of bisdethiobis(methylthio)acetylaranotin (**6**)



X-ray crystal structure analysis of bisdethiobis(methylthio)acetylaranotin (6): colorless block crystal of $C_{24}H_{26}N_2O_8S_2$. Space group P 2(1), a = 9.4623(12) Å, b = 12.9379(16) Å, c = 10.97900(13) Å, α = 90.00°, β = 110.758(2), γ = 90.00°, V_ 1256.8(2) Å^3, Z = 2, crystal size 0.50 × 0.47 × 0.46 mm^3. A total of 3094 unique reflections (2θ < 50°) were collected using graphite monochromated MoKa (λ = 0.71073 Å) on a CCD area detector diffractometer. The structure was solved by direct methods (SHELXS-97) and expanded using Fourier techniques. The final cycle of full-matrix least squares refinement was based on 3094 unique reflections (2θ < 50°) and 325 variable parameters and converged with unweighted and weighted agreement factors of R = 0.0442, R_w = 0.0995 and R_1 = 0.0387 for *I>2sigma(I)* data. Crystallographic data excluding structure factors for structure **6** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 725712. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: _44 (0) 1223336033 or e-mail: deposit@ccdc.cam.ac.uk]

Figure S2. The ^{1}H-NMR spectrum of alternaroside A (**1**) in d_6-DMSO

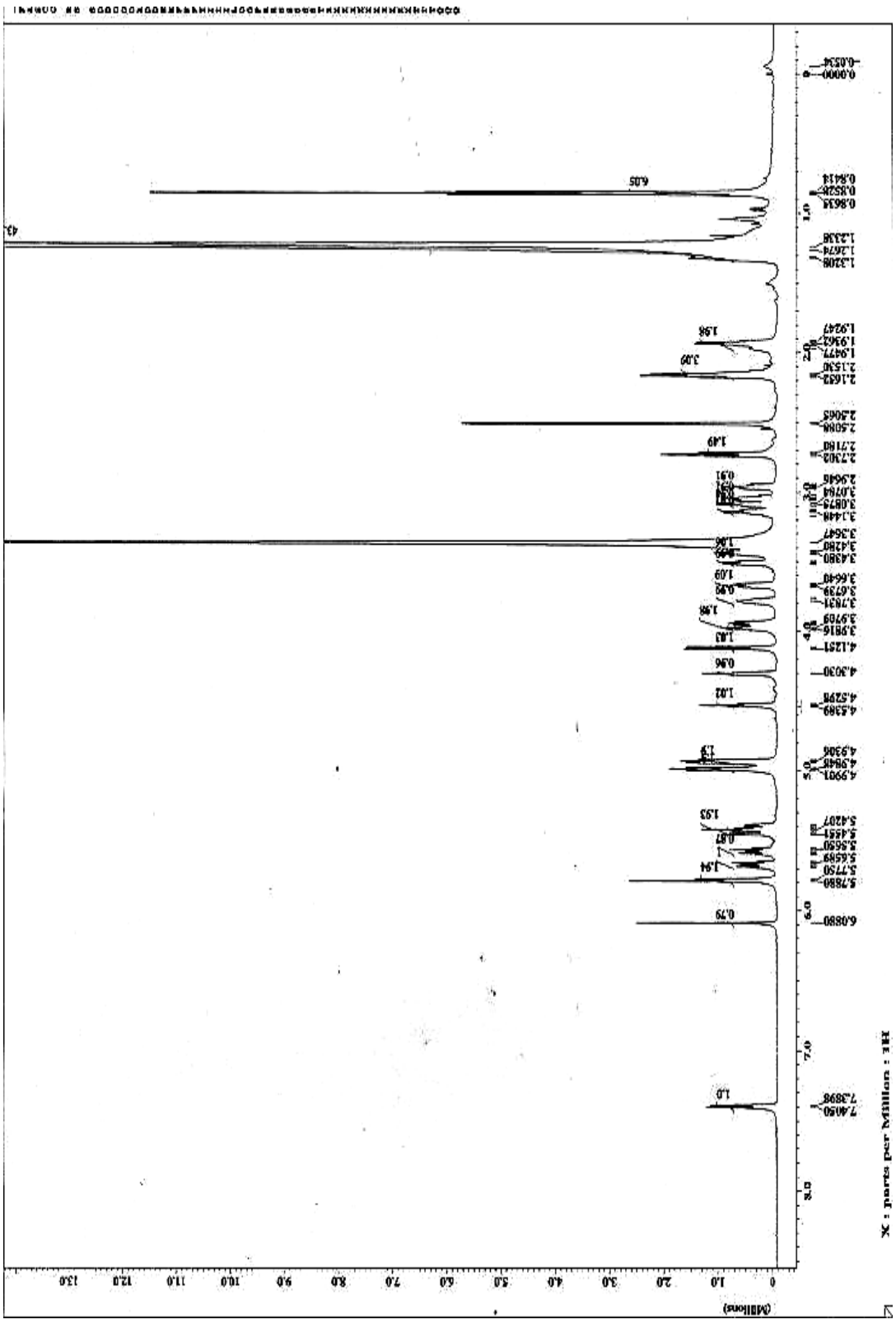


Figure S3. The ^{13}C-NMR spectrum of alternaroside A (**1**) in d_6-DMSO

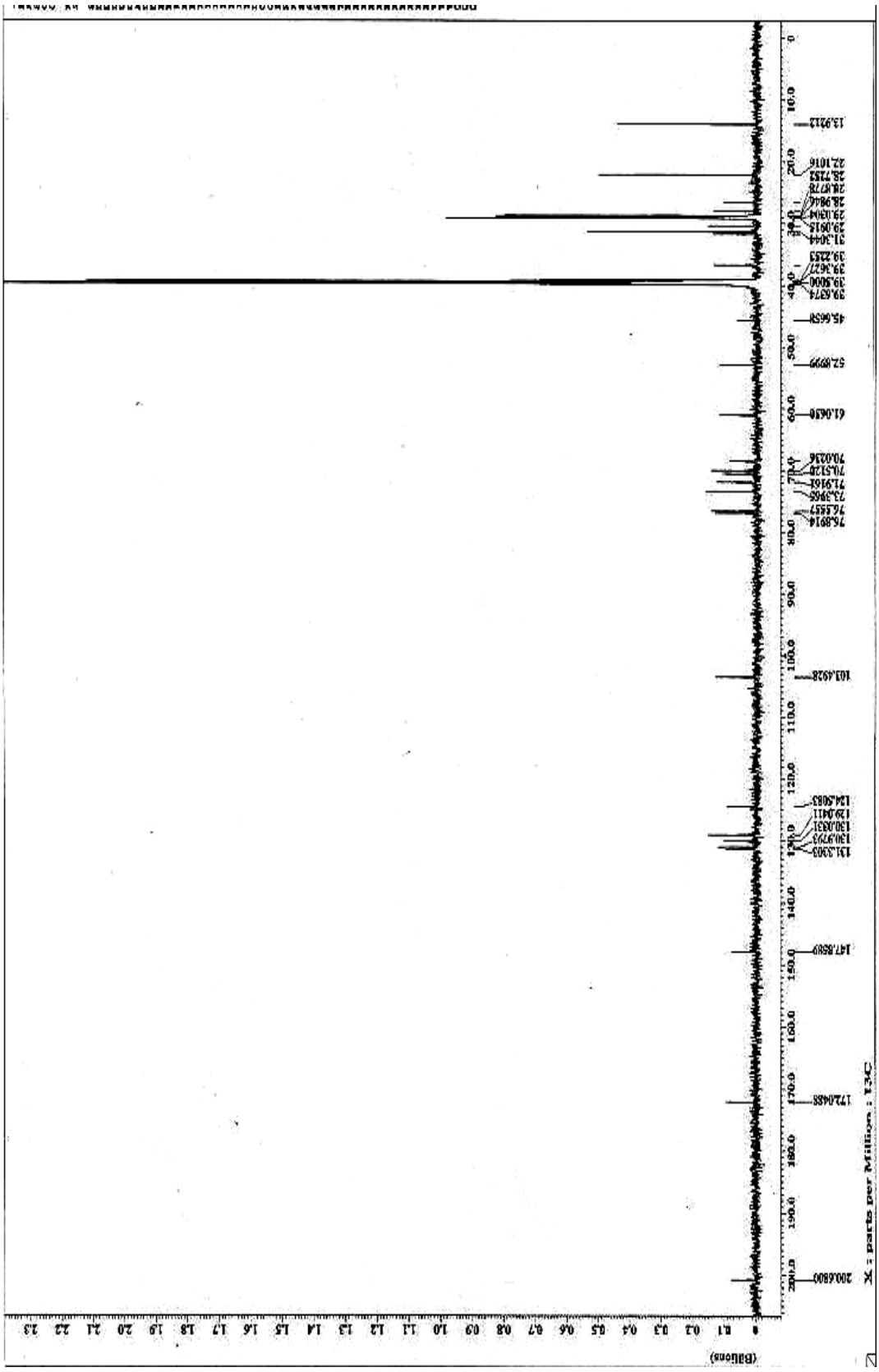


Figure S4. The ^{1}H-NMR spectrum of alternaroside B (**2**) in d_6-DMSO

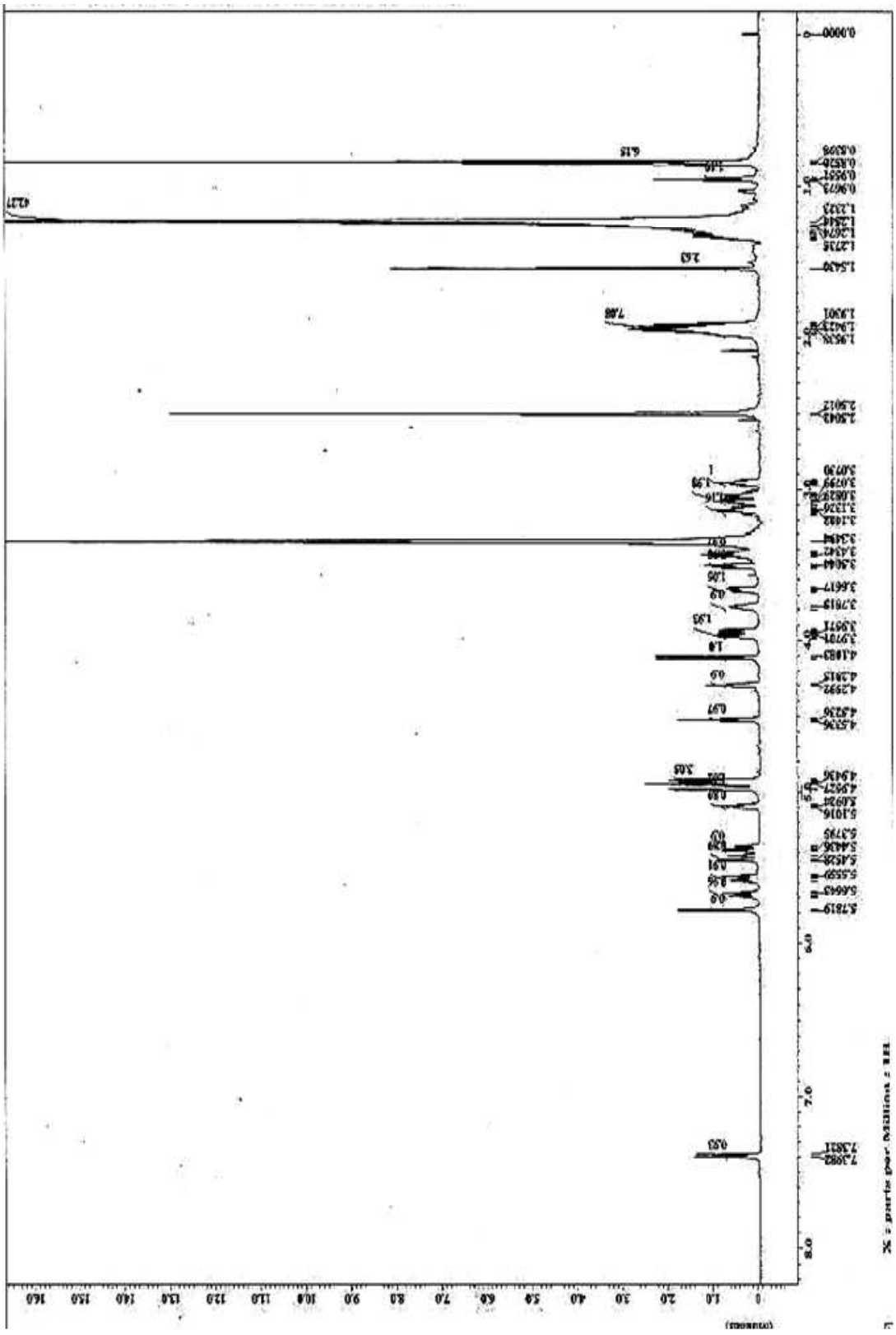


Figure S5. The ^{13}C-NMR spectrum of alternaroside B (**2**) in d_6-DMSO

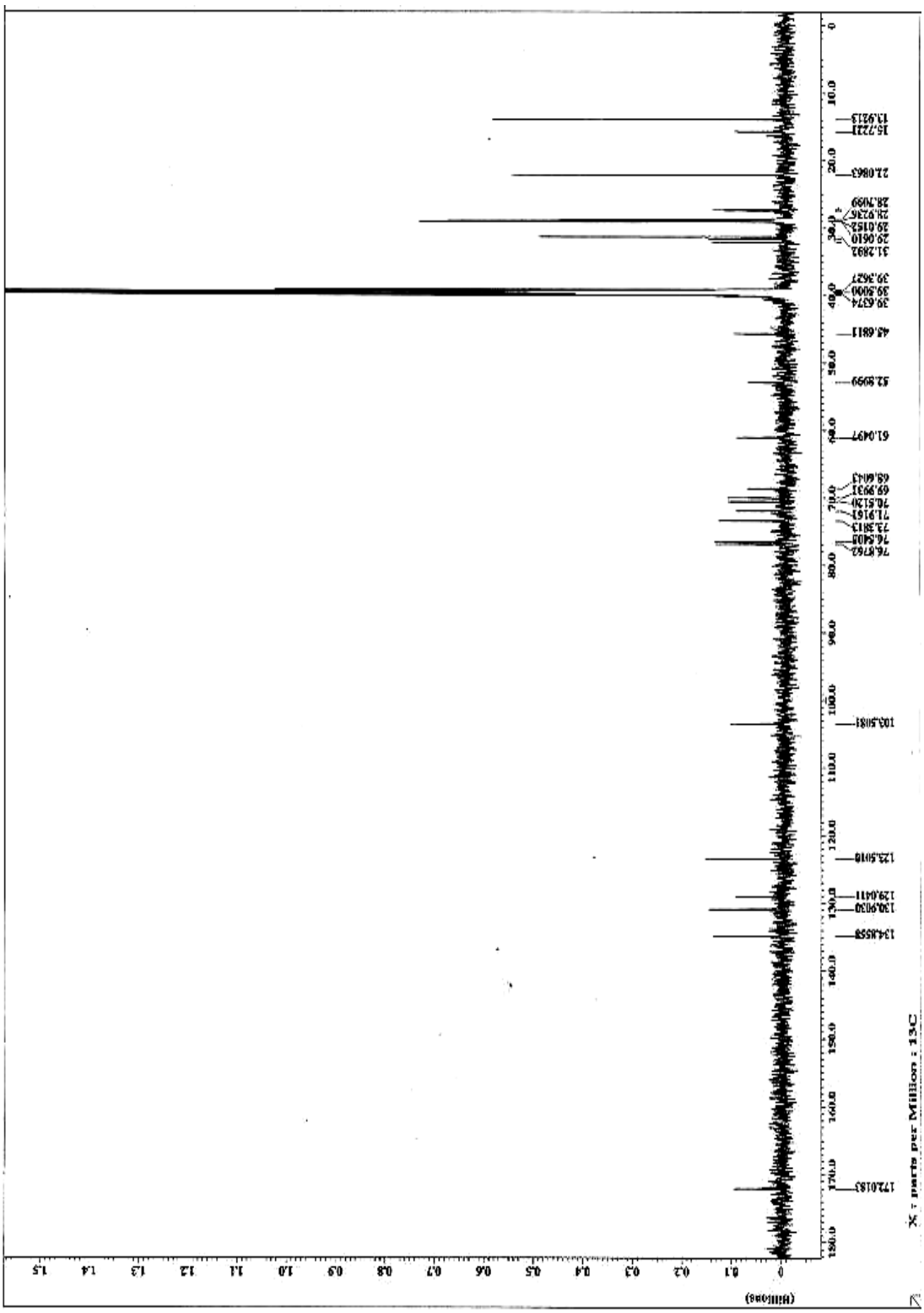


Figure S6. The ^{1}H-NMR spectrum of alternaroside C (**3**) in d_6-DMSO

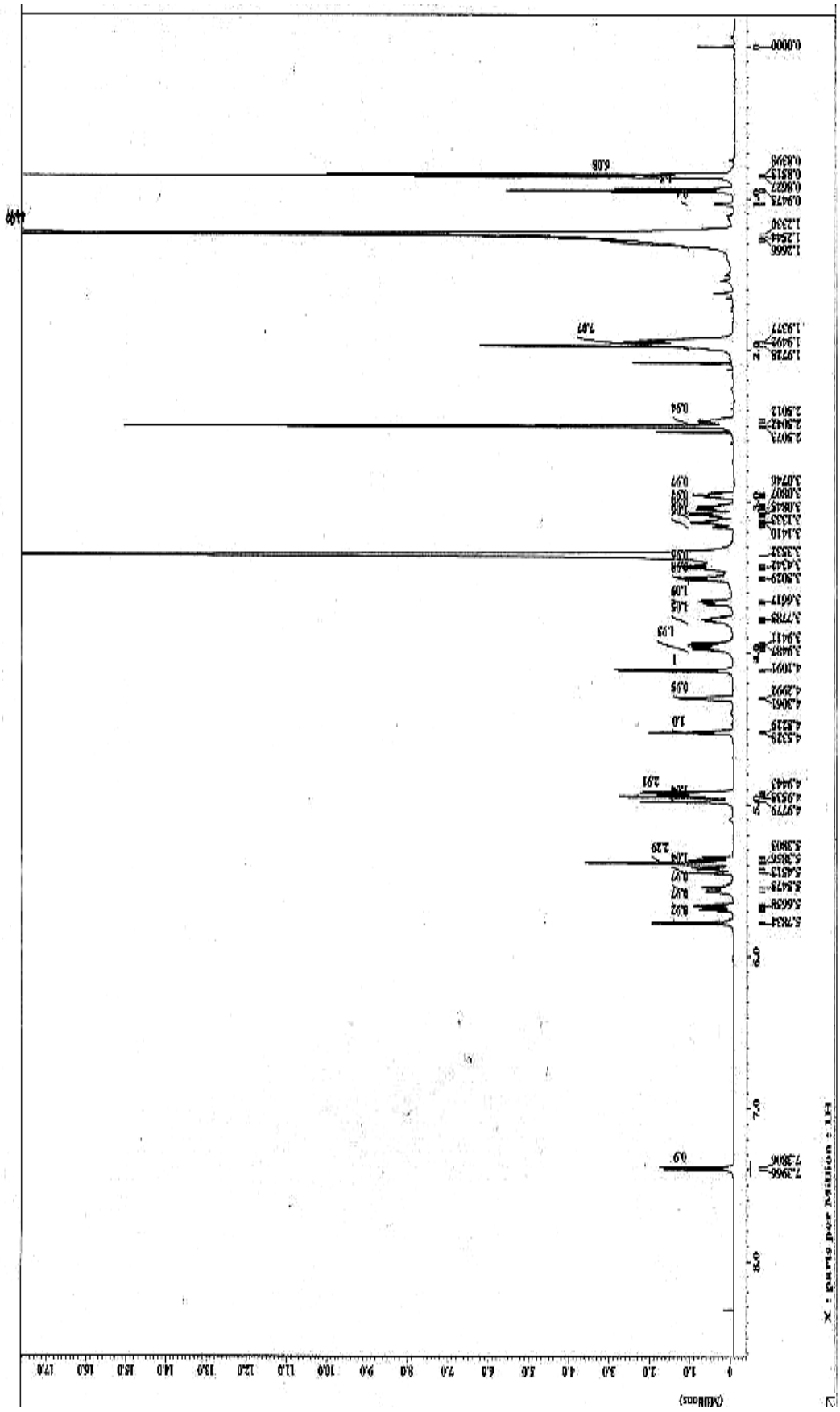


Figure S7. The ^{13}C-NMR spectrum of alternaroside C (**3**) in d_6-DMSO

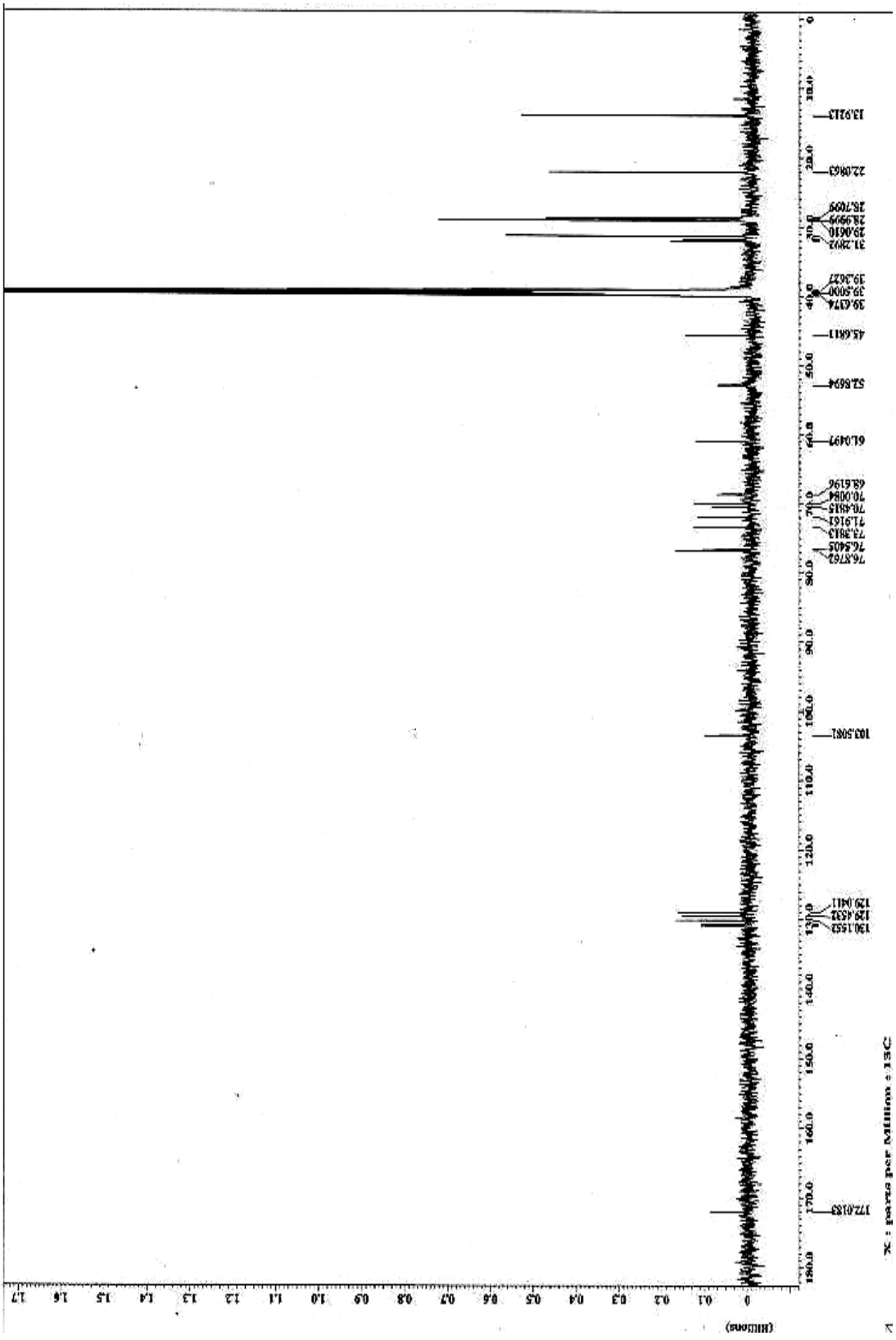


Figure S8. The ^{1}H-NMR spectrum of alternarosin A (**4**) in d_6-DMSO

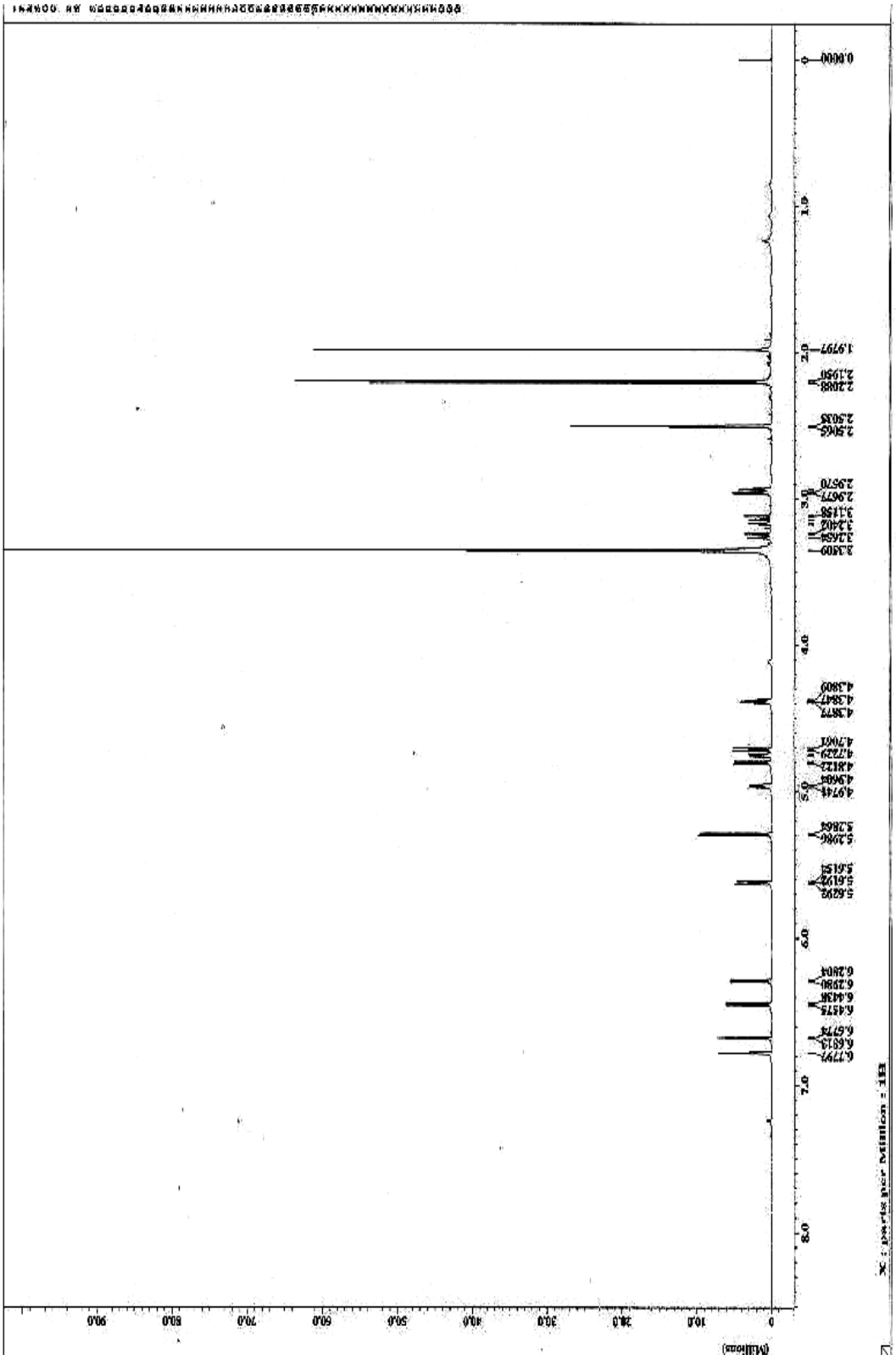


Figure S9. The ^{13}C-NMR spectrum of alternarosin A (**4**) in d_6-DMSO

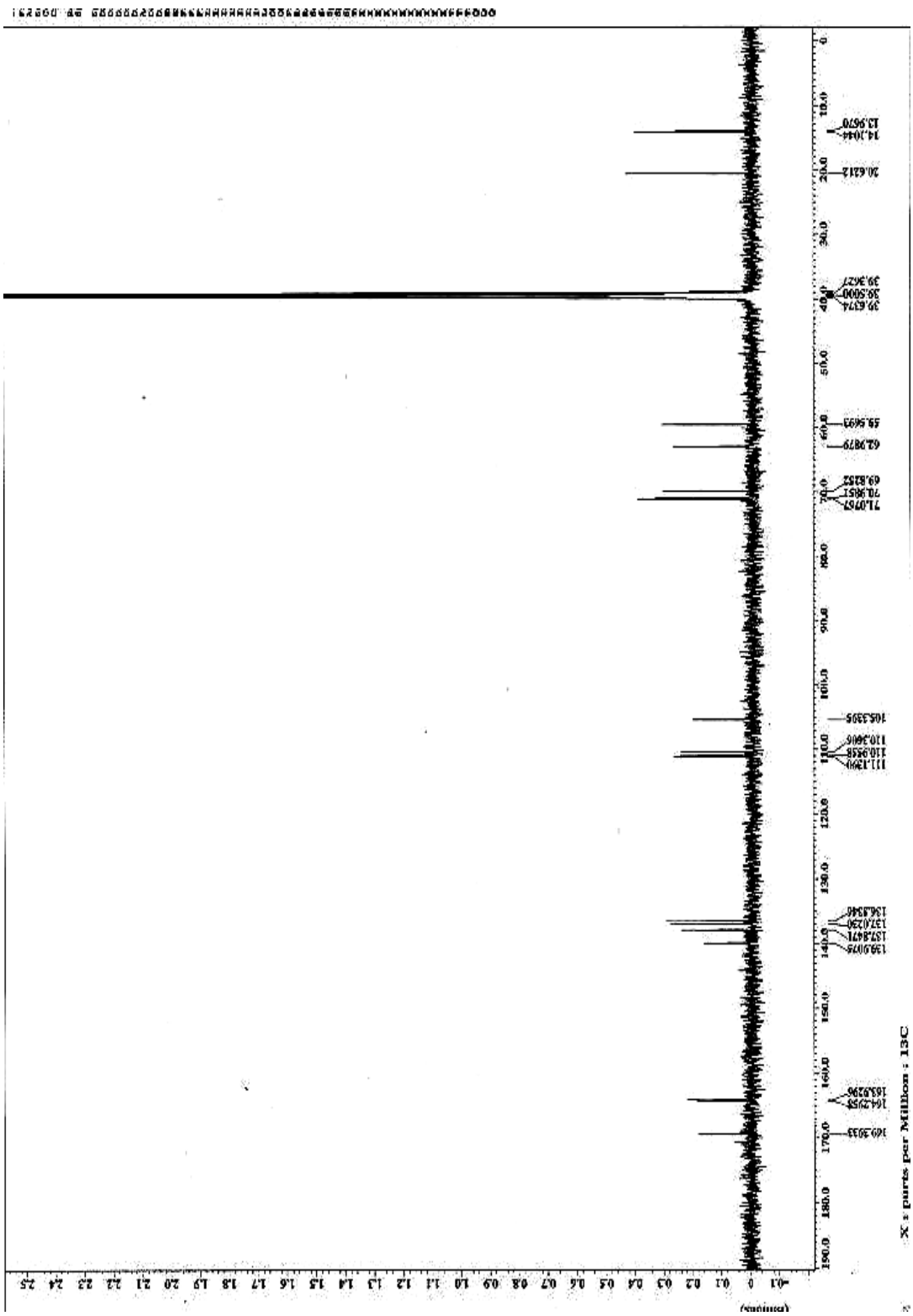


Figure S10. The positive-ion ESIMS/MS spectrum of alternaroside A (**1**)

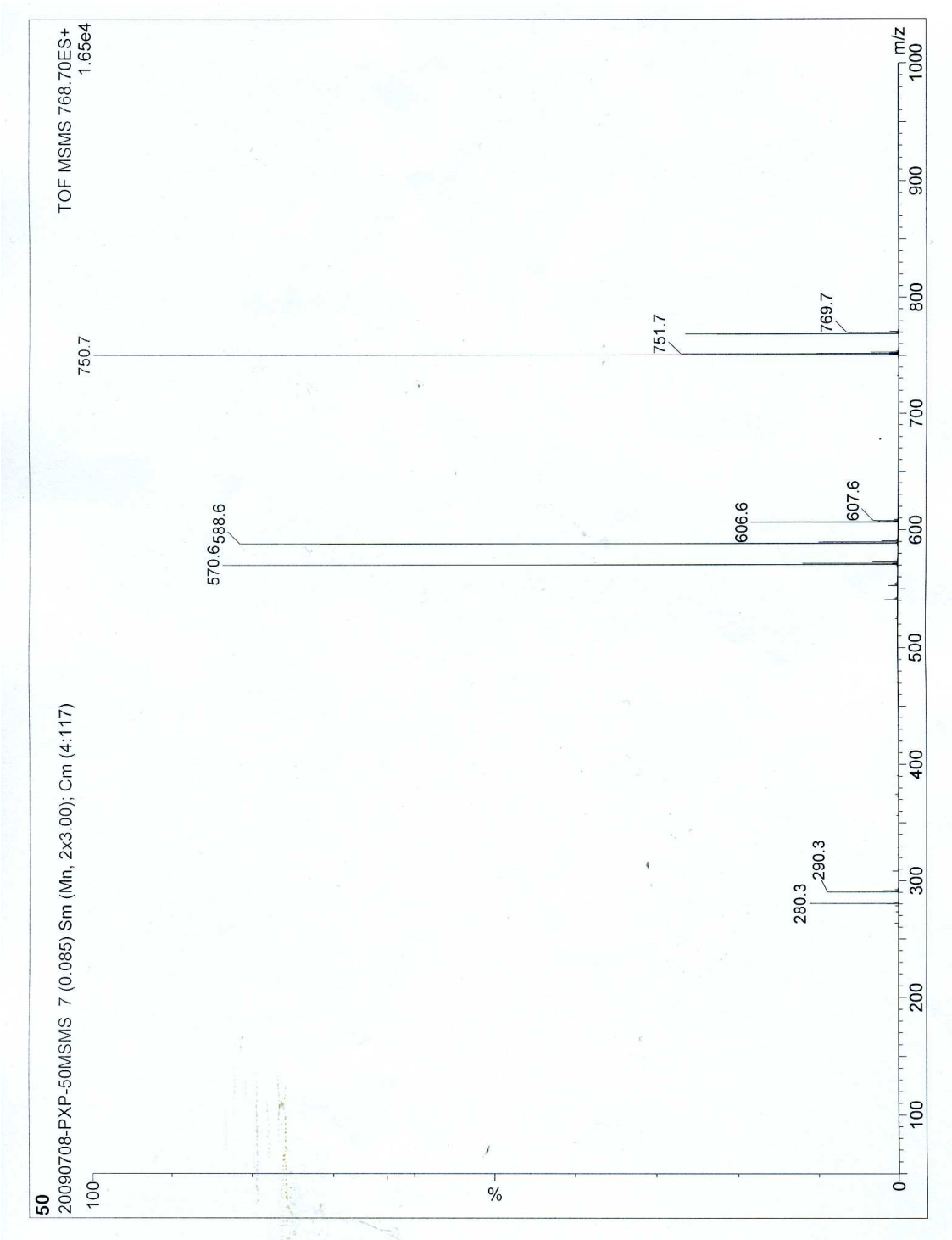


Figure S11. The positive-ion ESIMS/MS spectrum of alternaroside B (**2**)

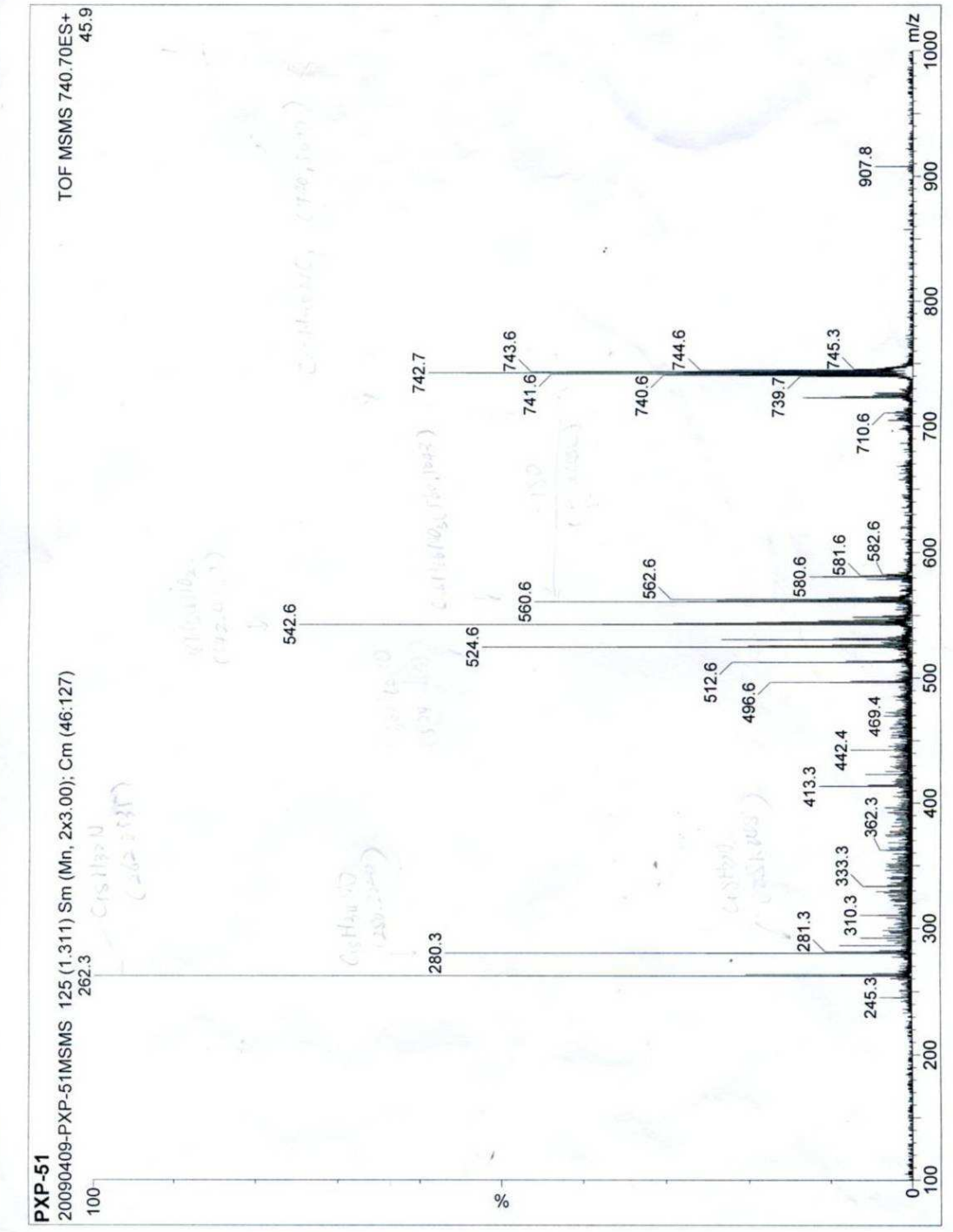


Figure S12. The positive-ion ESIMS/MS spectrum of alternaroside C (**3**)

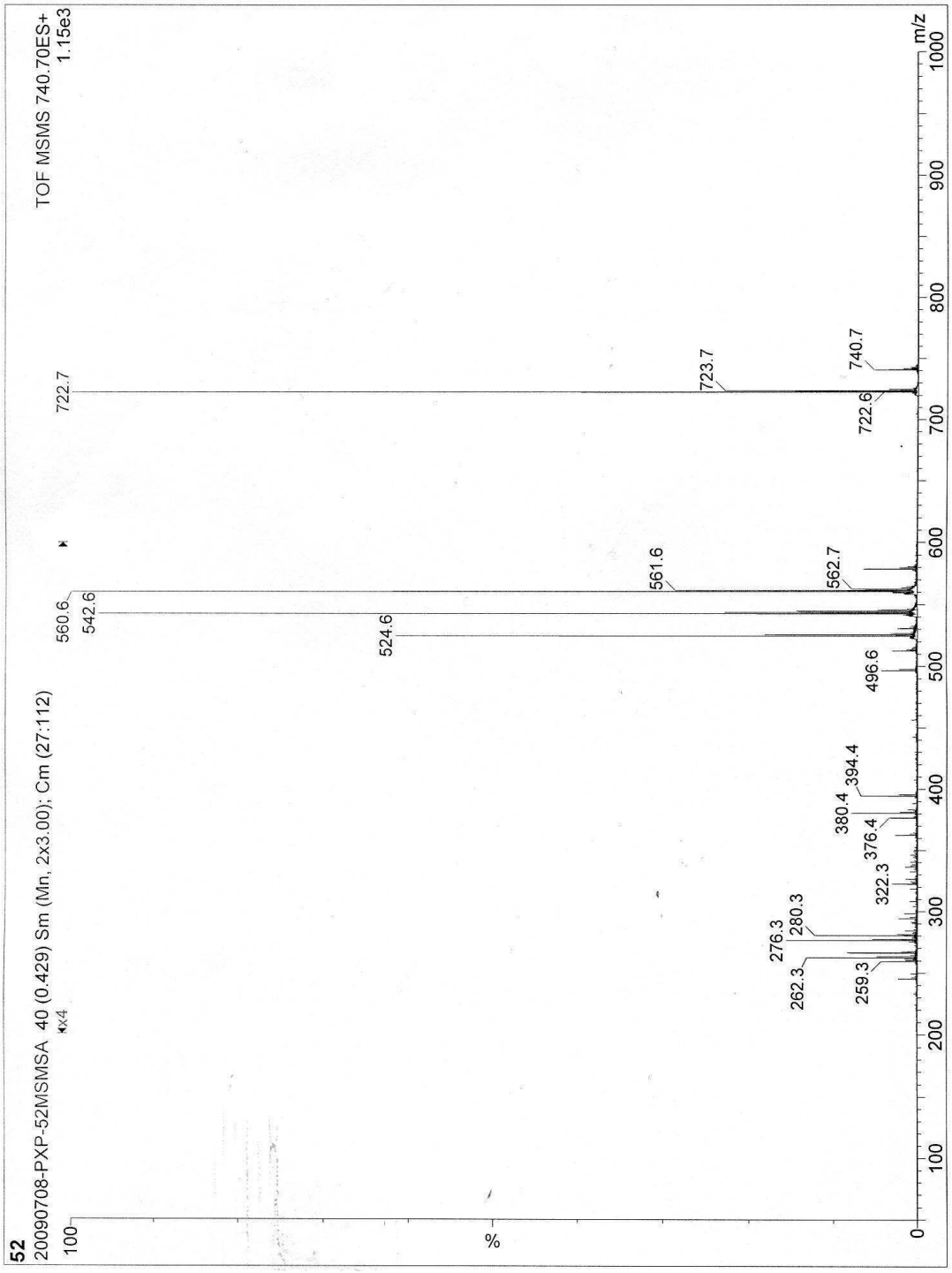


Figure S13. GC/MS of methyl 2-hydroxyoctadec-3-enoate (t_R 16.81 min) from

methanolysis of alternaroside A (**1**)

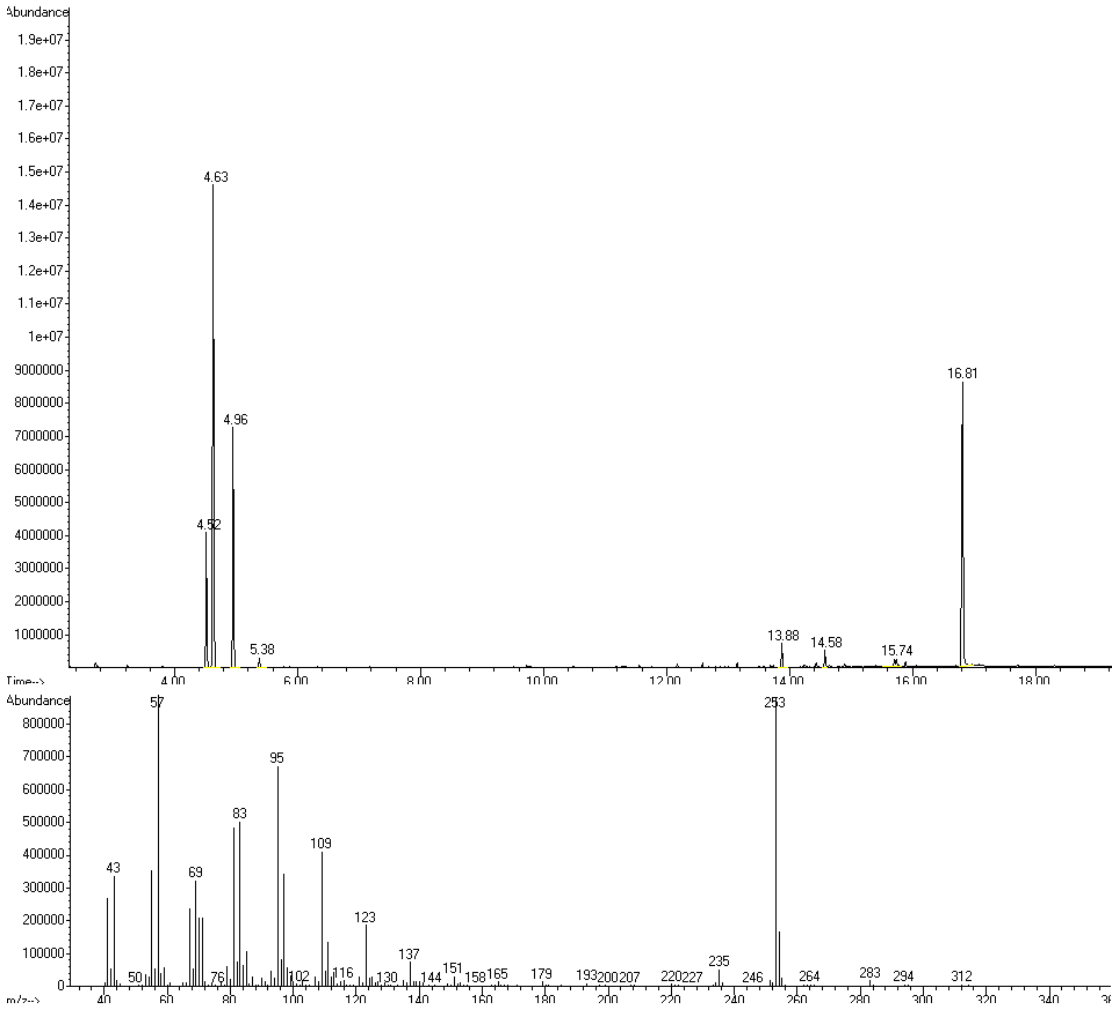


Figure S14. GC/MS of methyl 2-hydroxyoctadec-3-enoate (t_R 16.81 min) from

methanolysis of alternaroside B (**2**)

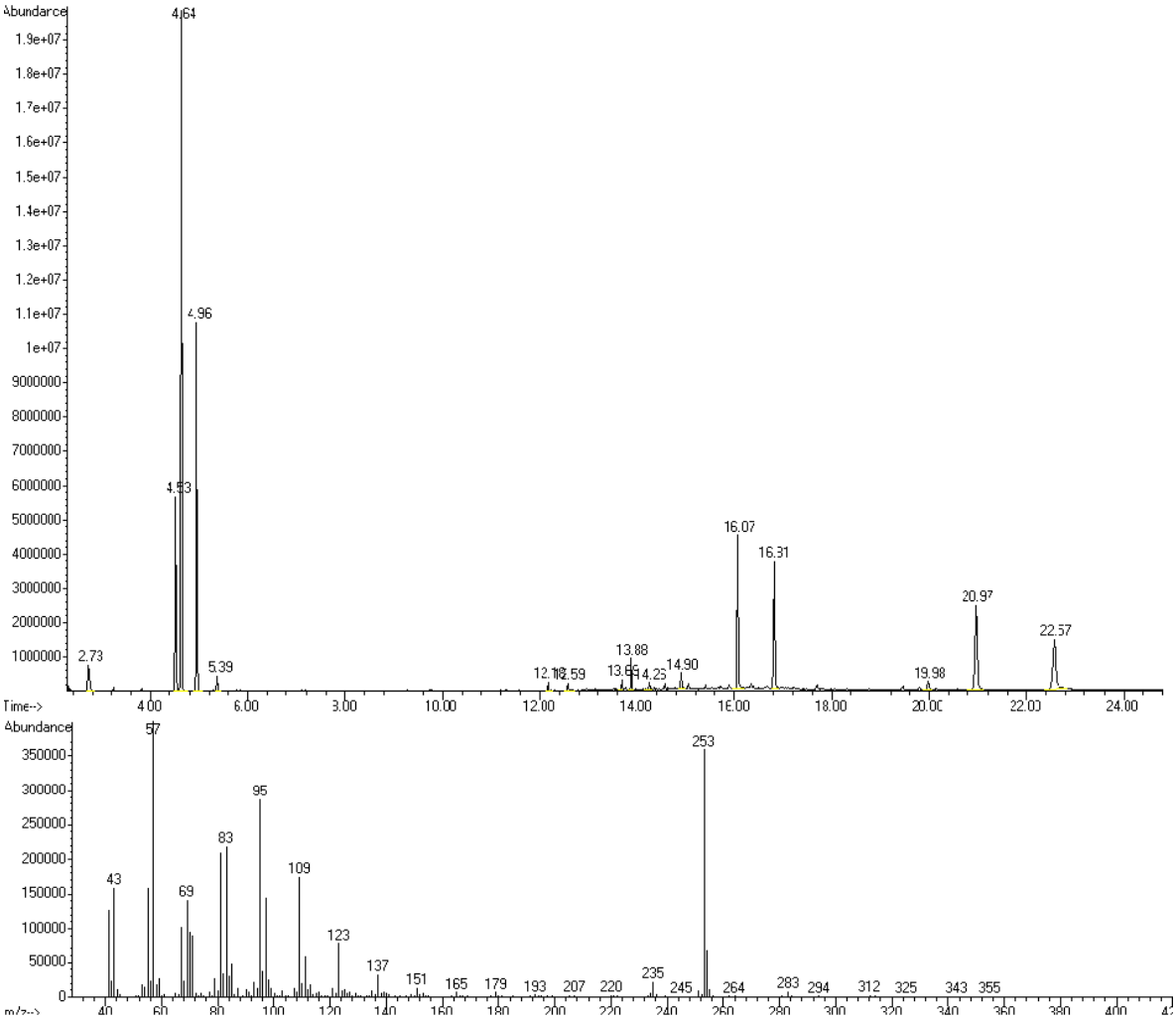


Figure S15. GC/MS of methyl 2-hydroxyoctadec-3-enoate (t_R 16.81 min) from

methanolysis of alternaroside C (**3**)

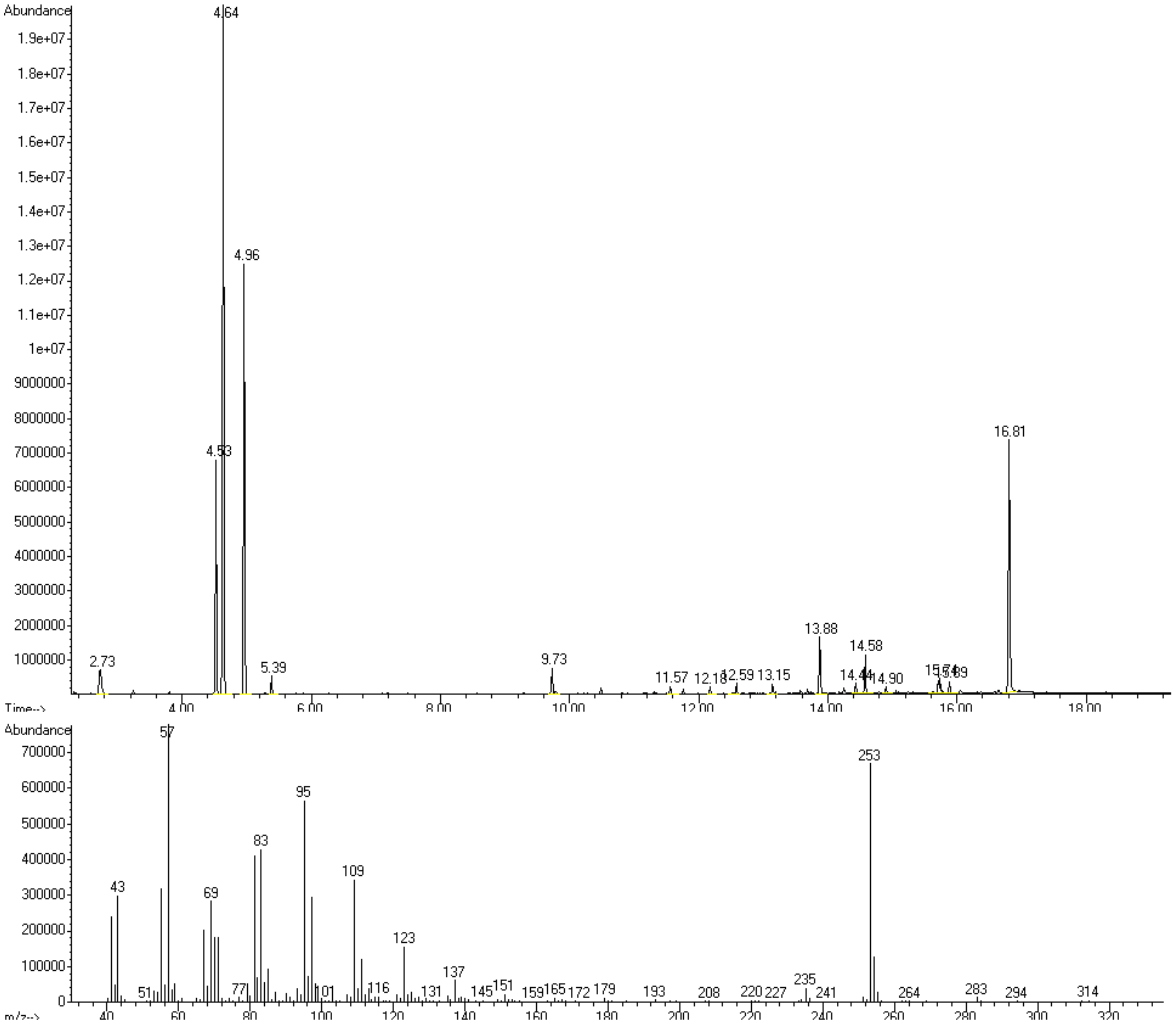


Figure S16. Elucidation by positive-ion ESIMS/MS of alternaroside A (**1**)

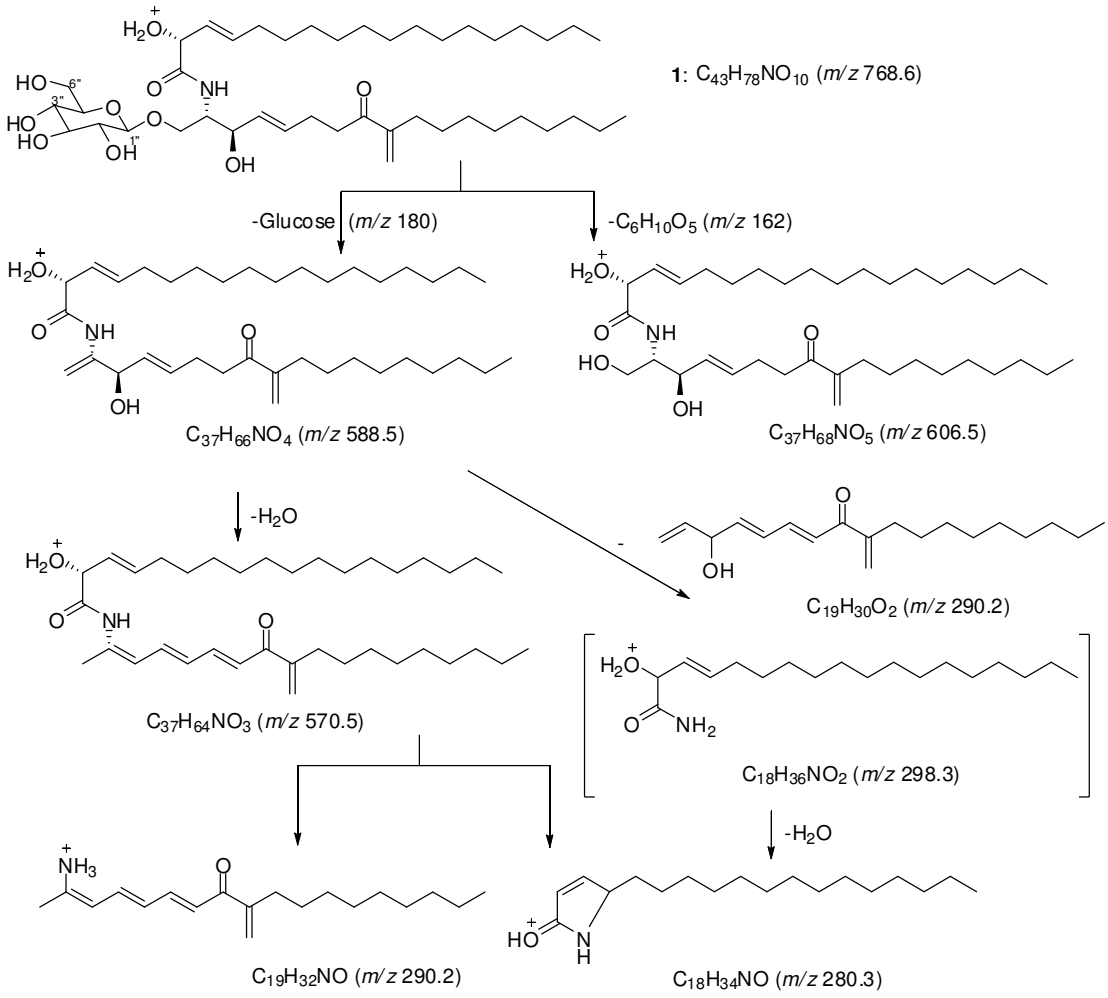


Figure S17. Elucidation by positive-ion ESIMS/MS of alternaroside B (**2**)

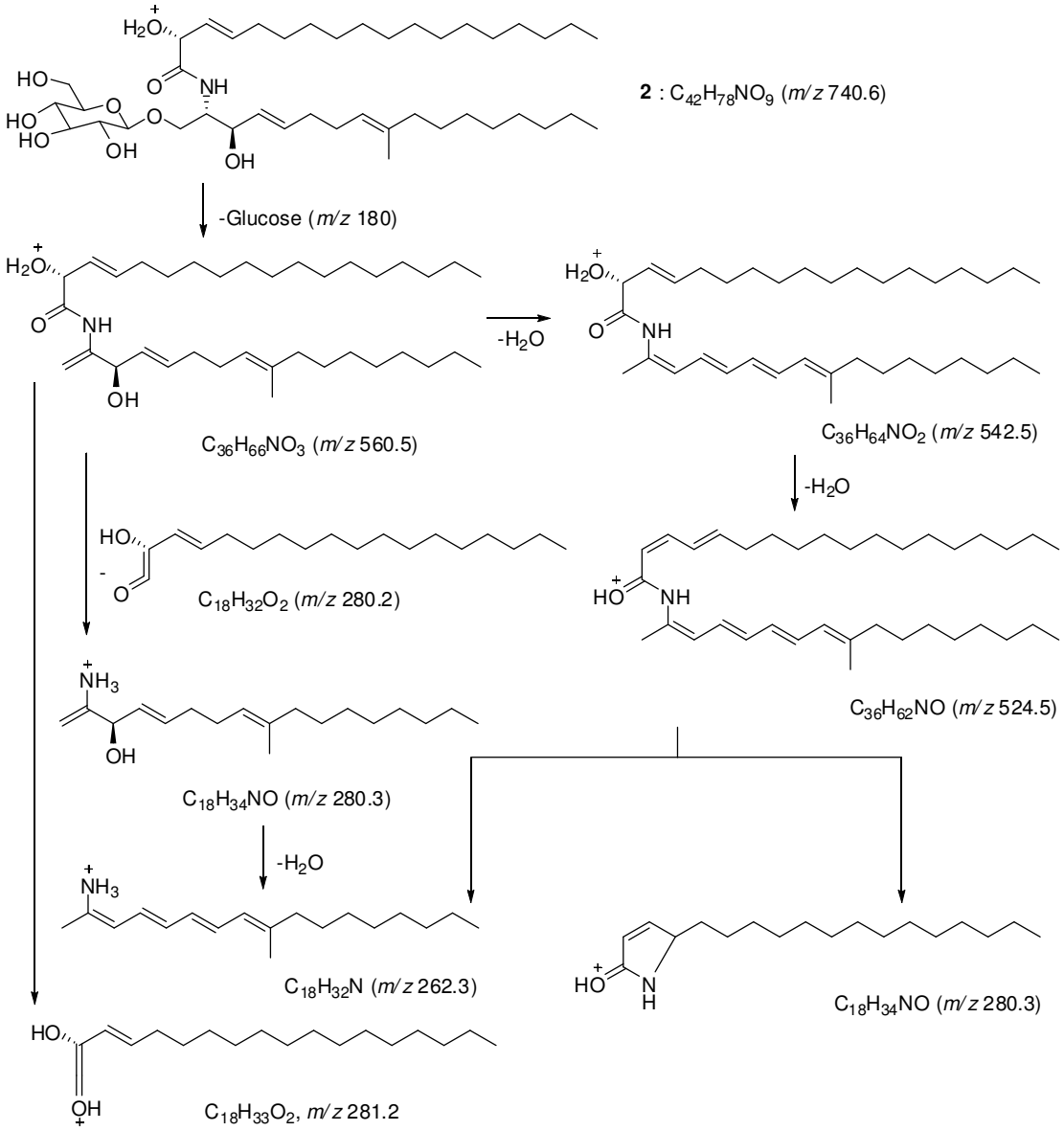
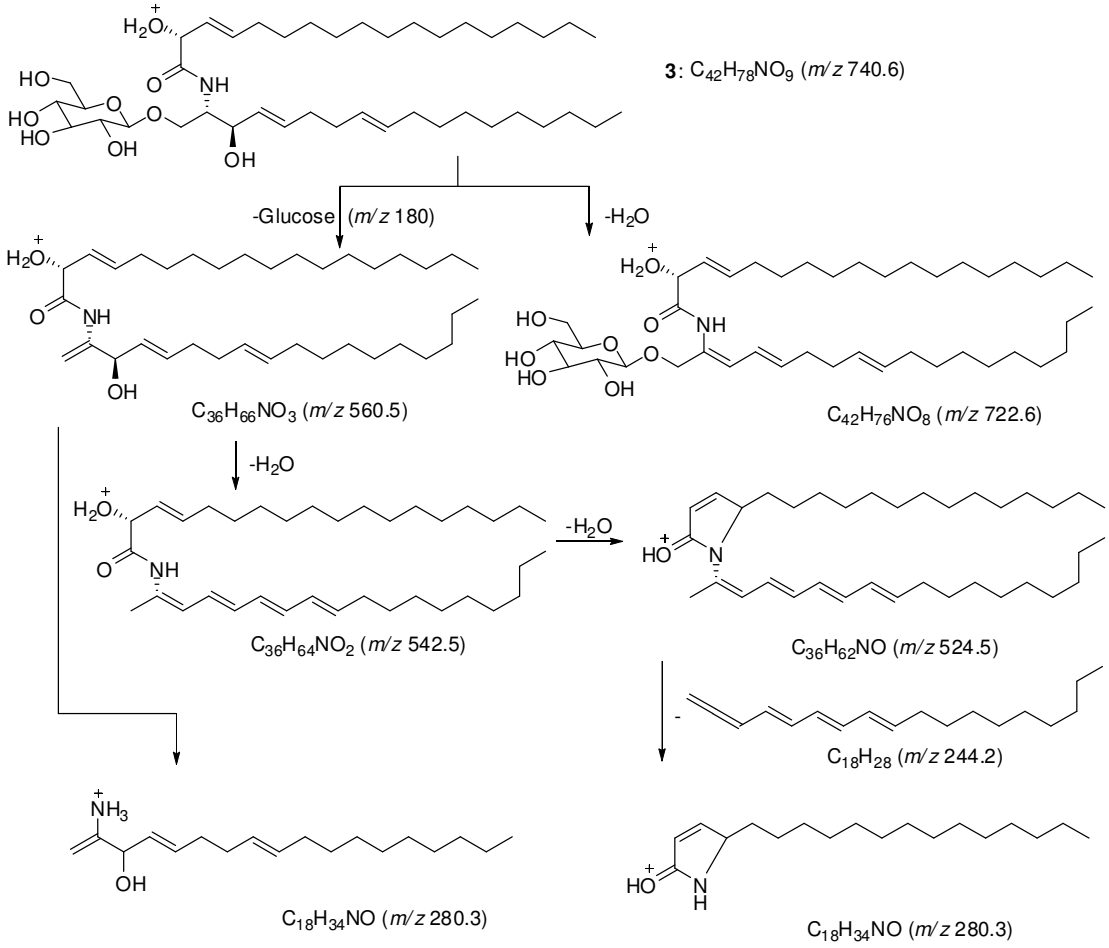


Figure S18. Elucidation by positive-ion ESIMS/MS of alternaroside C (**3**)


3: C42H78NO9 (m/z 740.6)
-Glucose (m/z 180)
-H2O
C36H66NO3 (m/z 560.5)
C42H76NO8 (m/z 722.6)
-H2O
C36H64NO2 (m/z 542.5)
-H2O
C36H62NO (m/z 524.5)
C18H28 (m/z 244.2)
C18H34NO (m/z 280.3)
C18H34NO (m/z 280.3)