Supporting Information

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3-*O*-Formyl-27-Hydroxyfusidic Acid: A New Metabolite of Fusidic Acid by *Cunninghamella echinulata*

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1. Experimental

1.1. General Procedures

Sodium fucidate was purchased from Leo Pharmaceutical Company (Ballerup, Denmark). IR and UV spectra were recorded using PerkinElmer IR and Shimadzu 60/PC ultraviolet spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were obtained on Bruker models AMX- 500 NMR spectrometer with standard pulse sequences operating at 500 MHz for ^{1}H, and 125 MHz for ^{13}C NMR, respectively. $CDCl_3$ as solvent and tetramethyl silane (TMS) as internal standard. HRESIMS was performed with a LCT Premier XE Micromass Waters spectrometer in the positive- ionization mode (Waters Corporation). The compounds were detected on Precoated silica gel 60 F_{254} plates (0.25 mm layer, E. Merck) using chloroform-methanol (5:1) or benzene-ethyl acetate-formic acid (3 mL:7 mL:1 drop) as mobile phases and visualized with *p*-anisaldehyde spray reagent after heating at 110 ˚C.

1.2. Microorganism Strain and Culture Conditions

Biotransformation studies were performed following previously reported procedures (Ibrahim et al., 2018). The fermentation medium (pH 6.0) is composed of 1% glycerol, 1% glucose, 0.5% peptone, 0.5%yeast extract, 0.5%NaCl, and 0.5% K_2HPO_4 in 1 L of distilled water and the medium was autoclaved at 121 ˚C for 15 min.

1.3. Large-scale Fermentation

Biotransformation process was carried out following the published procedures [1]. Two-week old slants of *C. echinulata* were used for preparing stage I cultures of which 5 ml was inoculated into new culture media to initiate stage II cultures. After 24 h, sodium fusidate was added and incubation continued for 6 days along with organism and substrate free cultures. Cultures were filtered after acidification with 10% HCl and the metabolites were extracted from the filtrate by chloroform which was dehydrated over anhydrous sodium sulphate. TLC was carried out as discussed in general procedures.

1.4. *Isolation of Metabolite* 2

A residue obtained by evaporating the chloroform extract (3.4 g) was loaded onto a silica gel column (300 g). Fractions of 125 ml were collected by using a gradient of ethyl acetate in benzene (0–60%) containing 0.2% formic acid which was increased to 0.4% starting from fraction 107. The residue of fractions 122-142 (300 mg), eluted with acidulated 60% ethyl acetate in benzene, was chromatographed again on a silica gel column (40 g) and 50 mL fractions were collected using a gradient of methanol in chloroform (0–10%). Fractions 37–38, eluted with 3% methanol, afforded compound **2** (10 mg).

1.5. Antimicrobial Activity

Minimum inhibitory concentration of compound **2** was determined using the National Committee of Clinical Laboratory Standard and ATCC strains.

Table S1: Antimicrobial activity testing of fusidic acid (**1**) and the isolated metabolite **2**

Microorganism	**MIC (μg/mL)**	
	1	**2**
Staphylococcus aureus (ATCC 25923)	0.38	1000
Escherichia coli (ATCC 25922)	-ve	2000
Pseudomonas aeruginosa (ATCC 15442)	-ve	-ve
Candida albicans (ATCC 10231)	12.5	2000

* -ve at the highest tested concentration (2000 μg/mL)

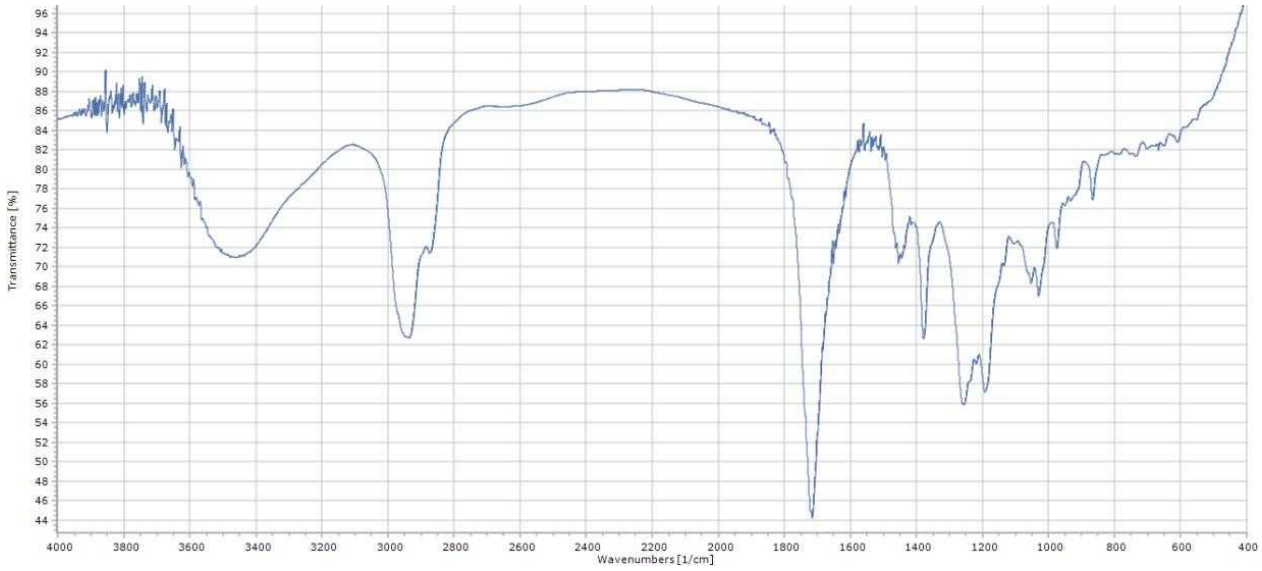

Figure S1: IR spectrum of 3-*O*-formyl-27-hydroxyfusidic acid (**2**)

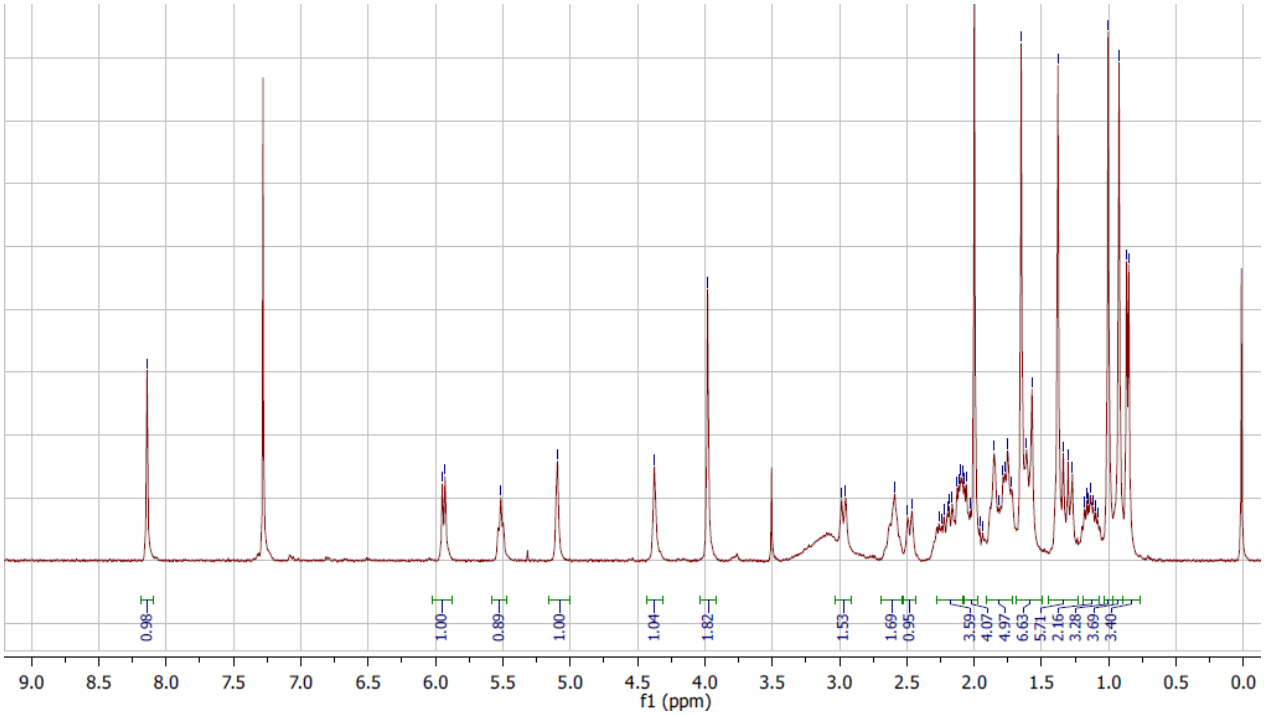

Figure S2: ^{1}H NMR spectrum of 3-*O*-formyl-27-hydroxyfusidic acid (**2**) ($CDCl_3$, 500 MHz)

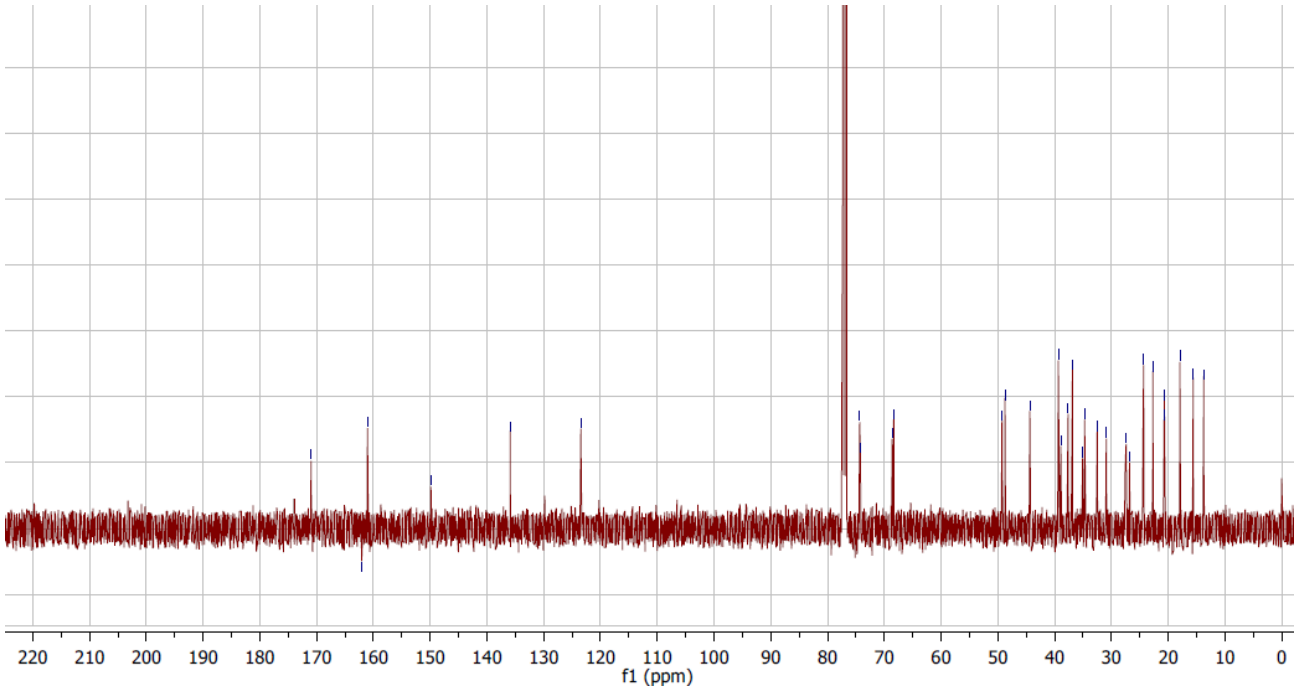

Figure S3: ^{13}C NMR spectra of 3-*O*-formyl-27-hydroxyfusidic acid (**2**) ($CDCl_3$, 125 MHz)

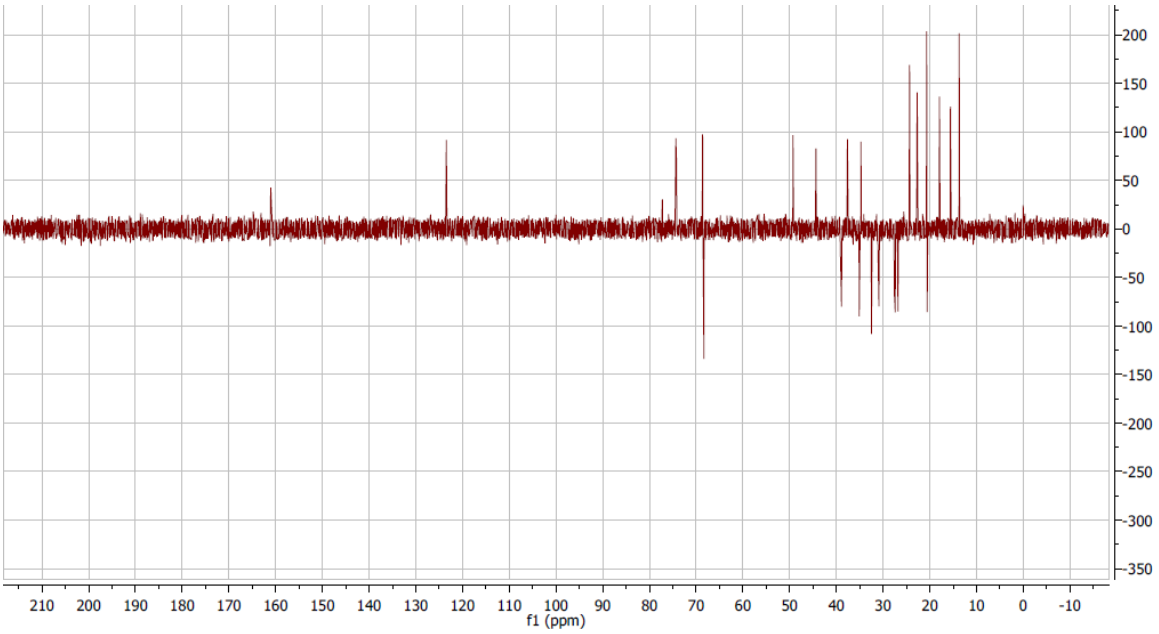

Figure S4: DEPT 135 spectrum of 3-*O*-formyl-27-hydroxyfusidic acid (**2**)

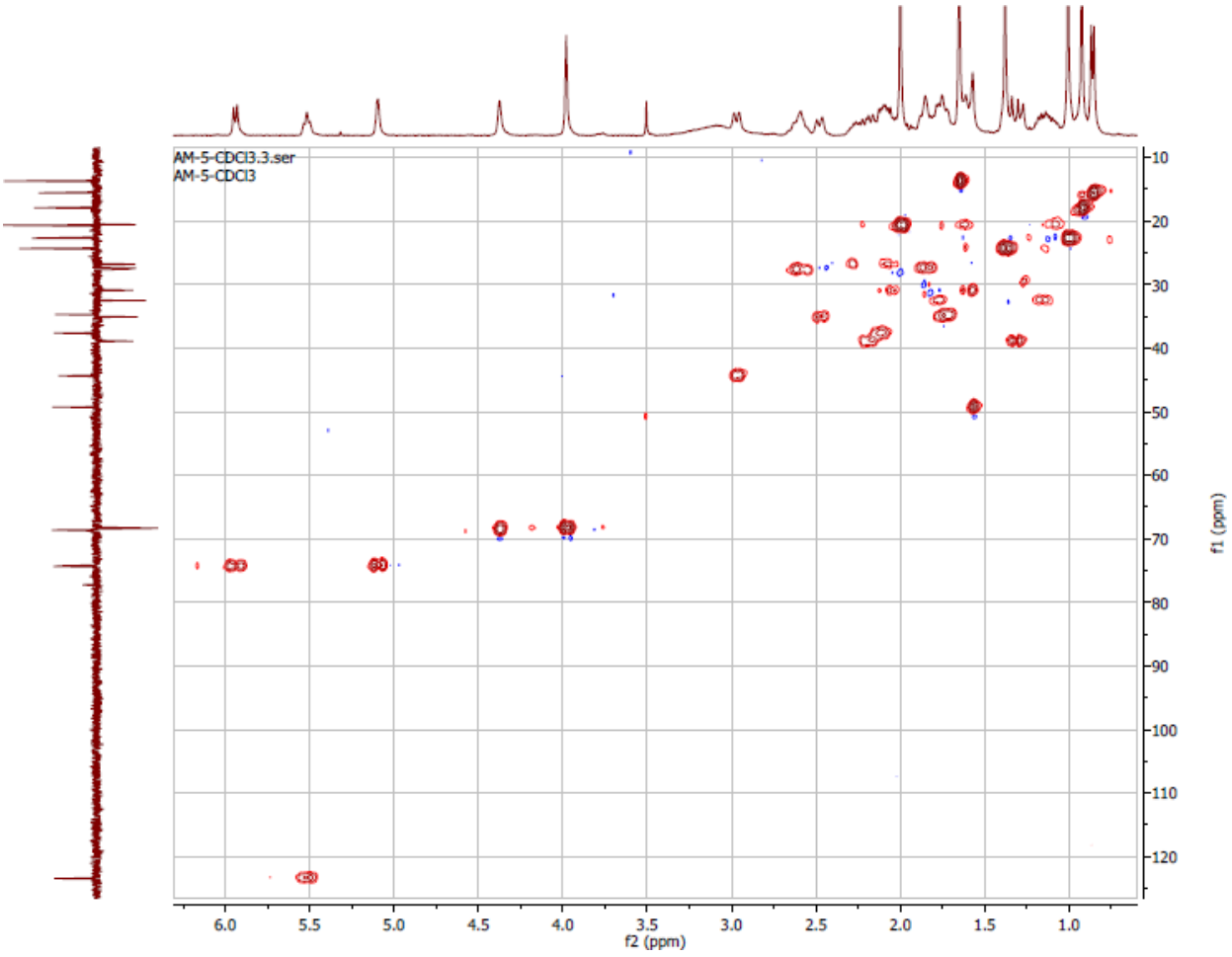

Figure S5: HMQC spectrum of 3-*O*-formyl-27-hydroxyfusidic acid (**2**)

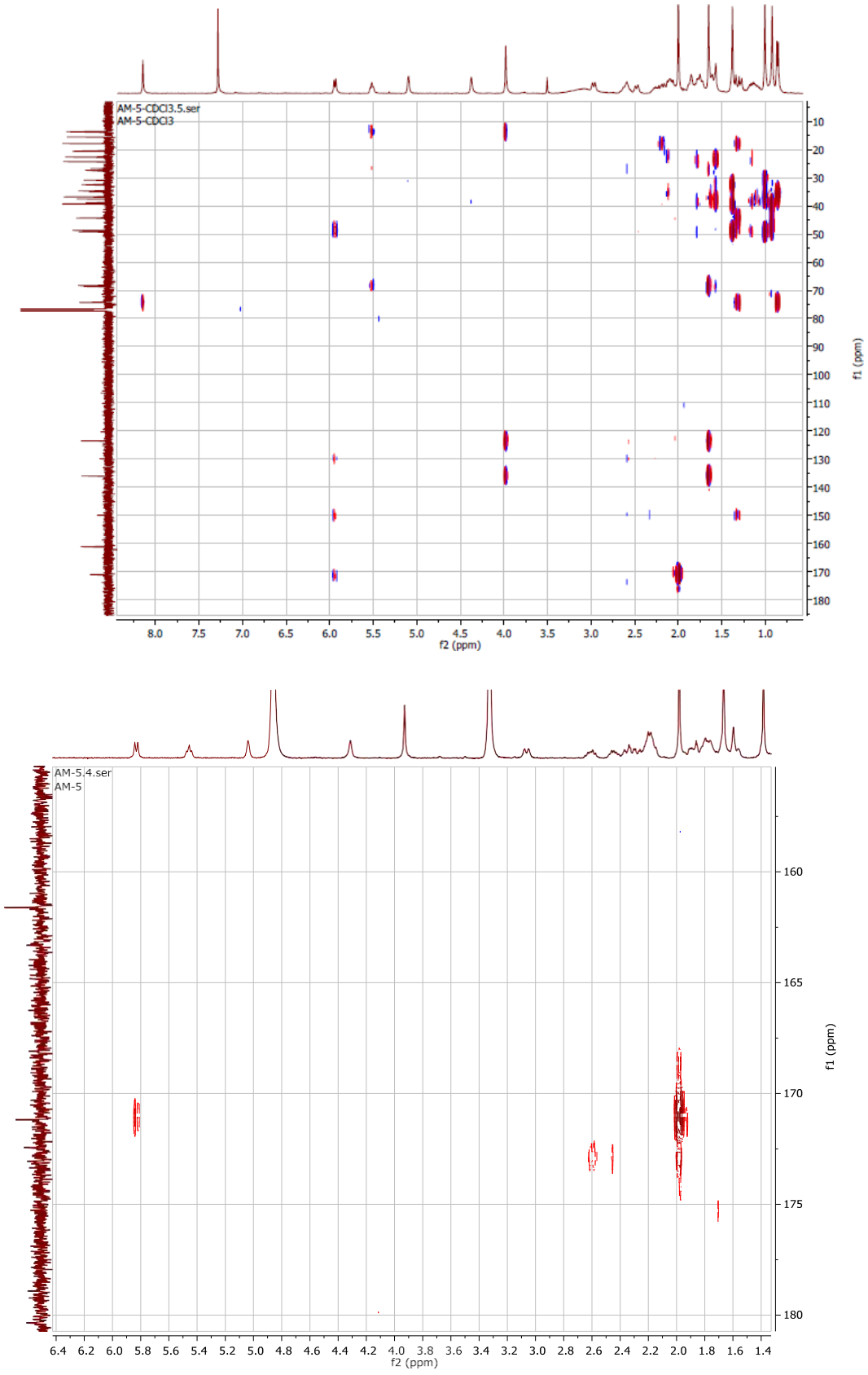

Figure S6: HMBC spectrum of 3-*O*-formyl-27-hydroxyfusidic acid (**2**) (in $CDCl_3$ [top], and methanol-d4 [bottom])

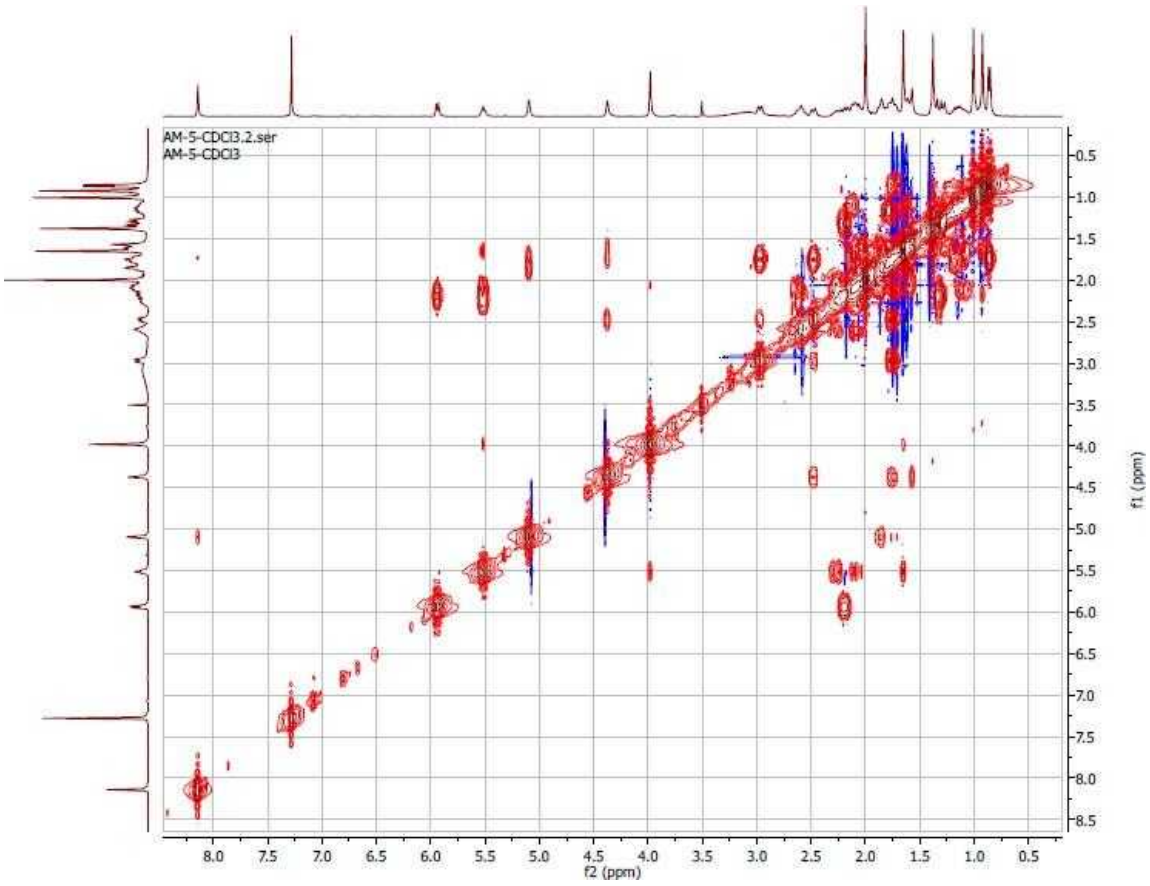

Figure S7: COSY spectrum of 3-*O*-formyl-27-hydroxyfusidic acid (**2**)

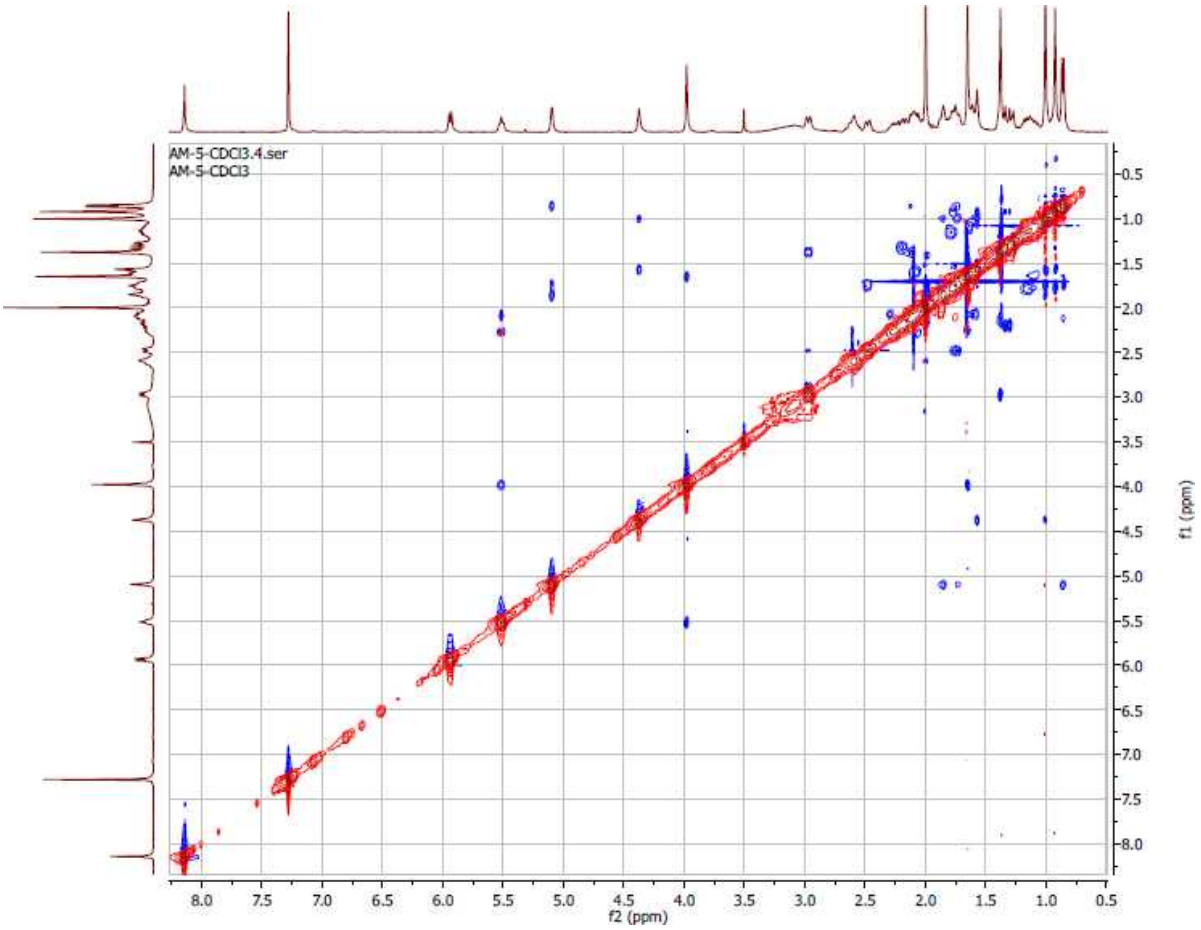

Figure S8: NOESY spectrum of 3-*O*-formyl-27-hydroxyfusidic acid (**2**)

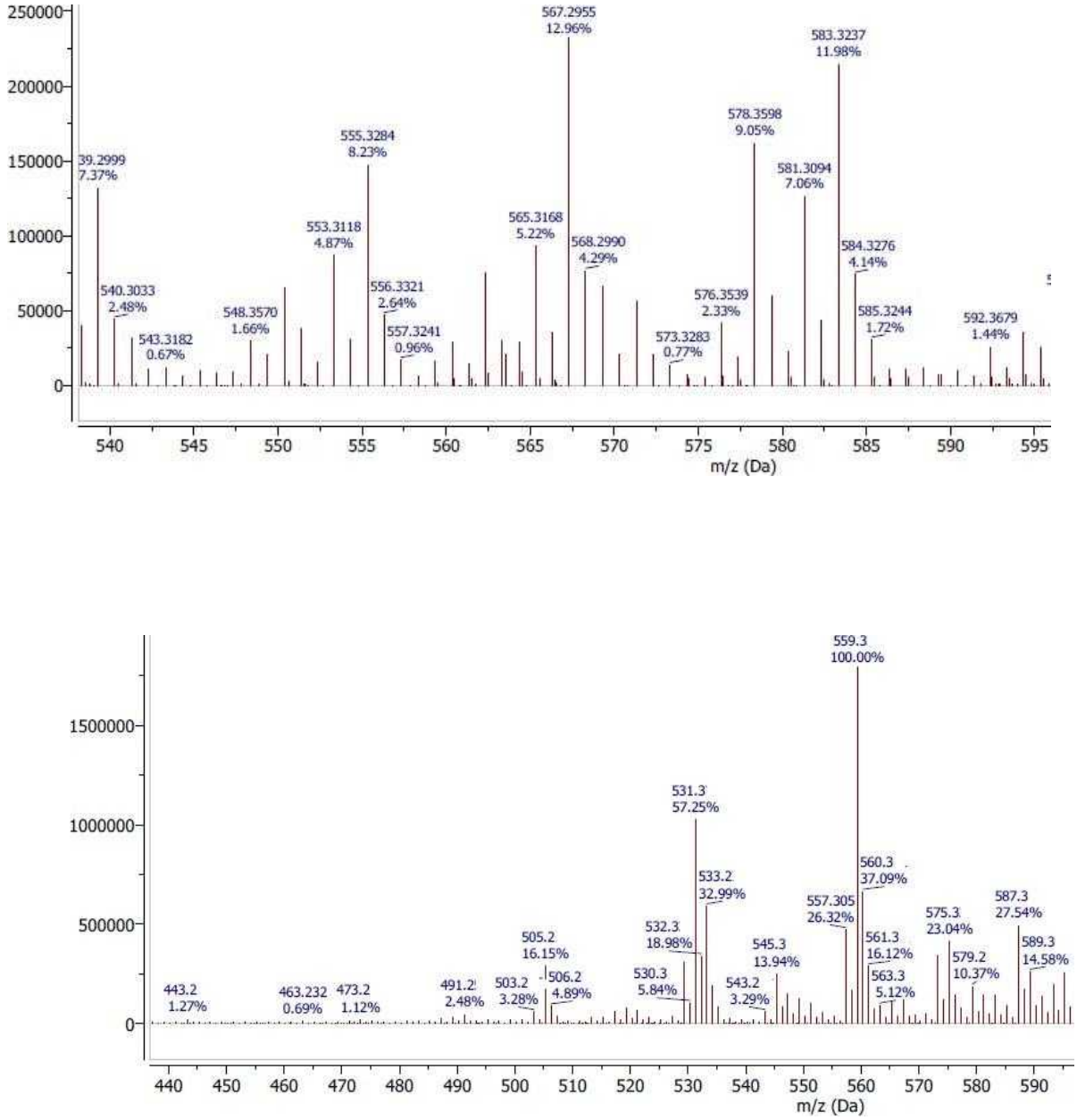

Figure S9: HRESIMS spectrum of 3-*O*-formyl-27-hydroxyfusidic acid (**2**) in the positive ion mode (top). Low resolution MS in the negative ion mode (bottom).

References

[1] A. S. Ibrahim, K. Elokely, D. Ferreira and A. E. Ragab (2018). Microbial oxidation of the fusidic acid side chain by *Cunninghamella echinulata*, *Molecules* **23**, 970-980.

