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Identification using LC-MS^n of Co-metabolites in the Biosynthesis of the Polyketide Toxin Mycolactone by a Clinical Isolate of *Mycobacterium ulcerans*

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Supplementary information:

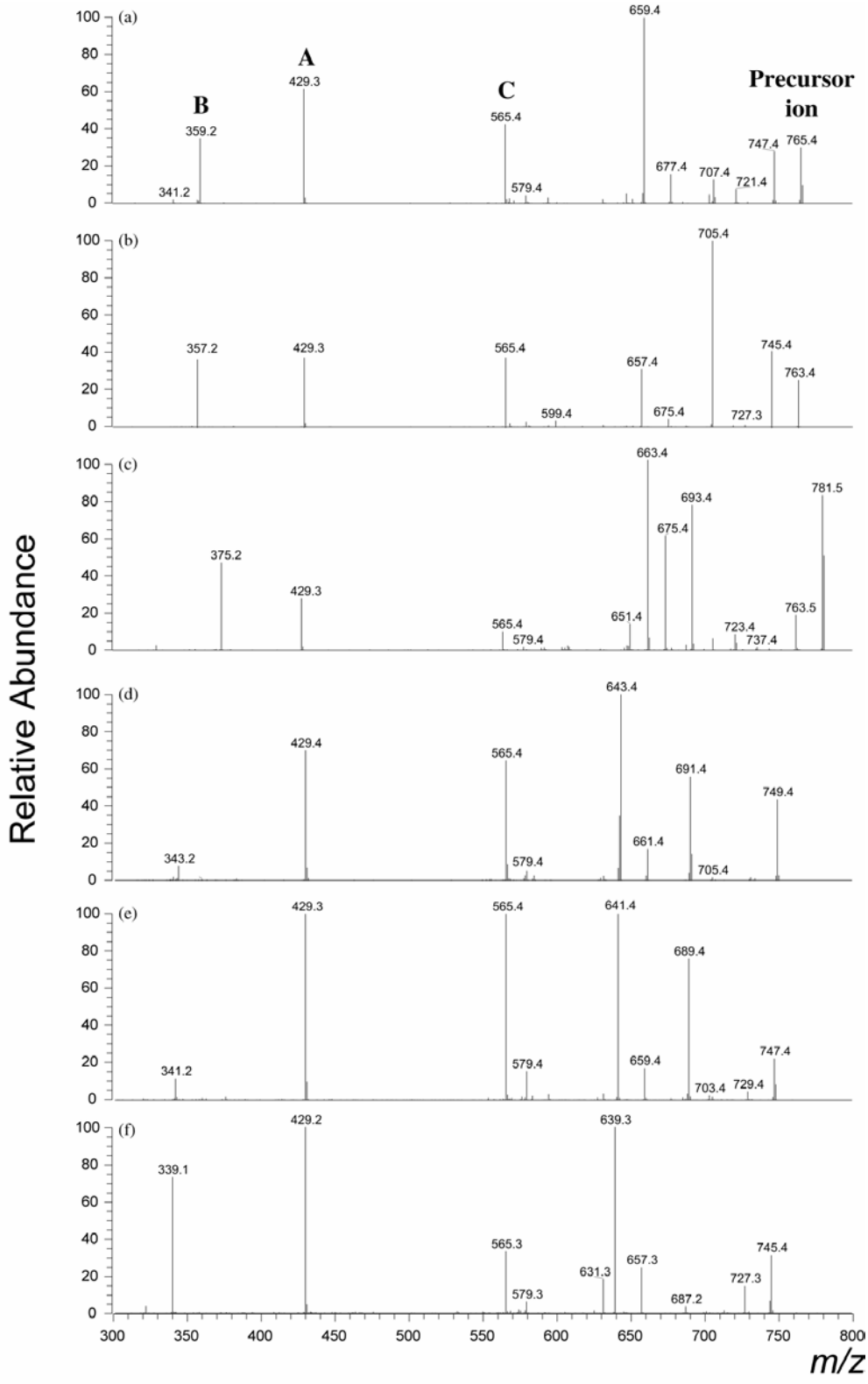

Figure S1. The ESI-CID-MS/MS spectra (LCQ) of mycolactone (a) and the 5 co-metabolites. The precursor ions (*m/z* 765.4, 763.4, 781.5, 749.4, 747.4 745.4) were isolated (±1 *m/z* isolation window) before fragmentation. Ions **A** and **C** (*m/z* 429 and 565) are present in all the spectra. Ion **B** varies in mass by the same amount as the precursor ion. This demonstrates that the structural alterations in the co-metabolites are all confined to within ion **B** – corresponding to the fatty acid side chain.

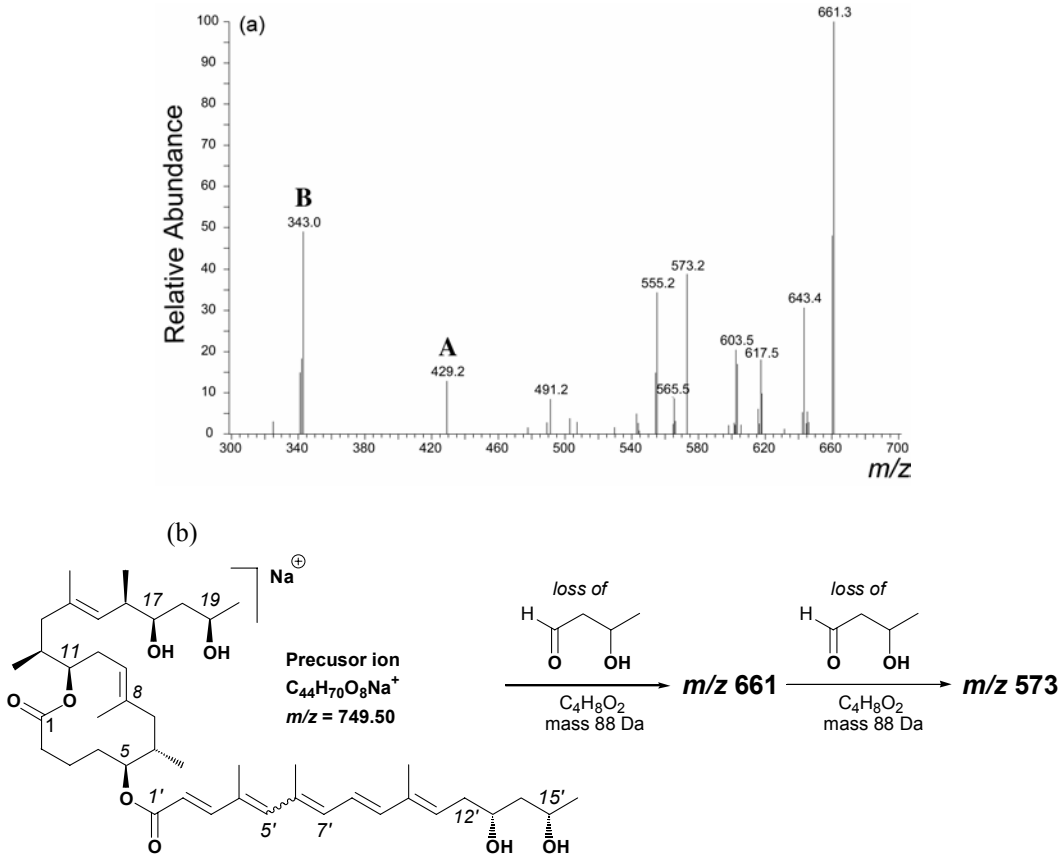

Figure S2. (a) MS^3 spectrum of *m/z* 661 from the MS/MS of *m/z* 749. Fragment ions **A** and **B** are labelled. (b) Scheme showing the losses of mass 88 ($C_4H_8O_2$) during the MS/MS of *m/z* 749 and the MS^3 of *m/z* 661. The first loss of mass 88 can either be C17 - C20 or C13' - C16'. A further loss of mass 88 then occurs in the MS^3 to form *m/z* 573, which confirms that C13' - C16' retains the same sub-structure as in mycolactone. The presence of both ions **A** and **B** in the spectrum demonstrate that the two losses of mass 88 can occur in parallel, therefore showing that *m/z* 661 is a mixture of two species.