Identification using LC-MSn of Co-metabolites in the Biosynthesis of the Polyketide Toxin Mycolactone by a Clinical Isolate of Mycobacterium ulcerans

Hui Hong, a Paul J. Gates, a James Staunton, a Tim Stinear, b Stuart T. Cole, b Peter F. Leadlay, a, b and Jonathan B. Spencer a, *

a Departments of Chemistry and Biochemistry, University of Cambridge, Cambridge, UK.
E-mail: jbs20@cam.ac.uk; Fax: 01223 336362; Tel: 01223 331696.
b Unité de Génétique Moléculaire Bactérienne, Institut Pasteur, Paris, France.

This submission was created using the RSC Communication Template (DO NOT DELETE THIS TEXT)
(LINE INCLUDED FOR SPACING ONLY - DO NOT DELETE THIS TEXT)

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 ($\text{C}_4\text{H}_8\text{O}_2$) 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 $\text{C}_{17} - \text{C}_{20}$ or $\text{C}_{13'} - \text{C}_{16'}$. A further loss of mass 88 then occurs in the MS$^3$ to form m/z 573, which confirms that $\text{C}_{13'} - \text{C}_{16'}$ 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.