

## Experimental procedures

† On-line ESI-LC-MS and ESI-LC-MS/MS analysis were carried out on an LCQ ion trap mass spectrometer (Thermo Finnigan, USA) as described previously<sup>9</sup>. High-resolution MS and MS/MS were performed on a BioApex II (4.7 Tesla) FTICR mass spectrometer (Bruker Daltonics, US) using CO<sub>2</sub> as collision gas.

‡ *M. ulcerans* strain MUAgy99 (isolated from a patient in Ghana in 1999) was cultured in Dubos broth at 30°C for eight weeks. Cells were harvested by centrifugation and acetone soluble lipids (including mycolactones) were extracted by the method of Rohr as previously described<sup>3</sup>. Mycolactones were prepared from MU128FXT as for MUAgy99 except that cells were harvested from cultures grown on solid egg-yolk agar medium for three weeks at 30°C.

§ Deuterium exchange experiments were performed essentially as previously described<sup>9</sup>. After exchange, the samples were analysed either by the LCQ or by the FTICR mass spectrometer. When FTICR-MS/MS experiments were performed, the fully deuterated parent ion was isotopically isolated for fragmentation.

**Table 1** Comparison of molecular formula, numbers of exchangeable protons, and of degree of unsaturation, in mycolactones from the African strain MUAgy99 and the frog pathogen MU128FXT strain.

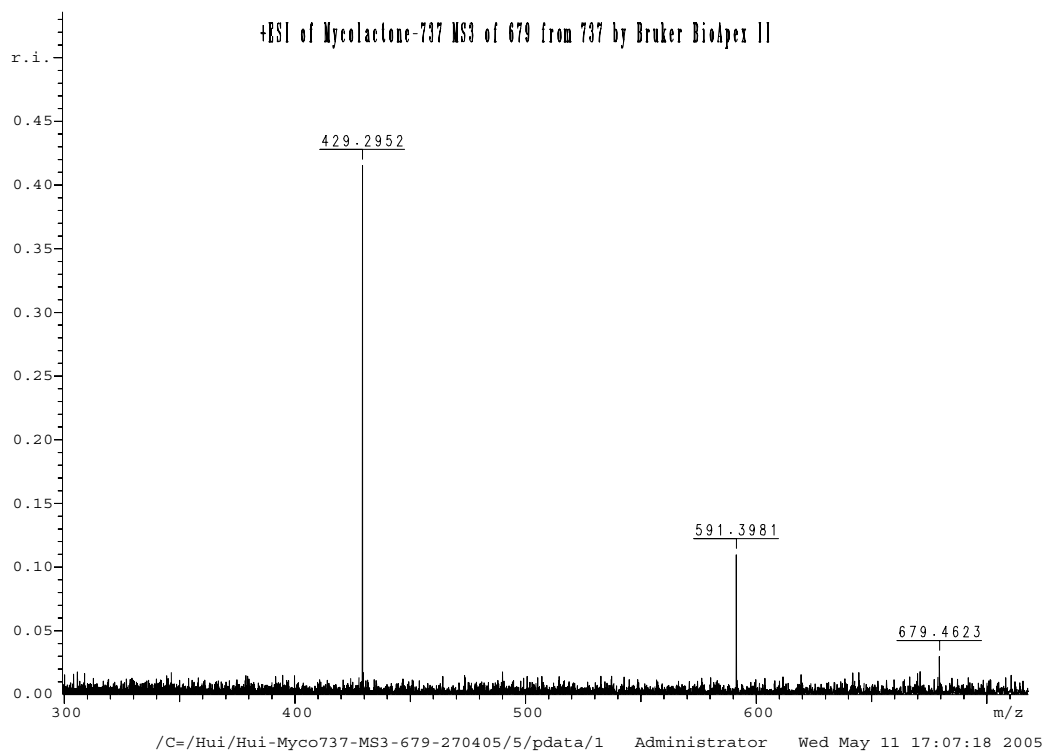
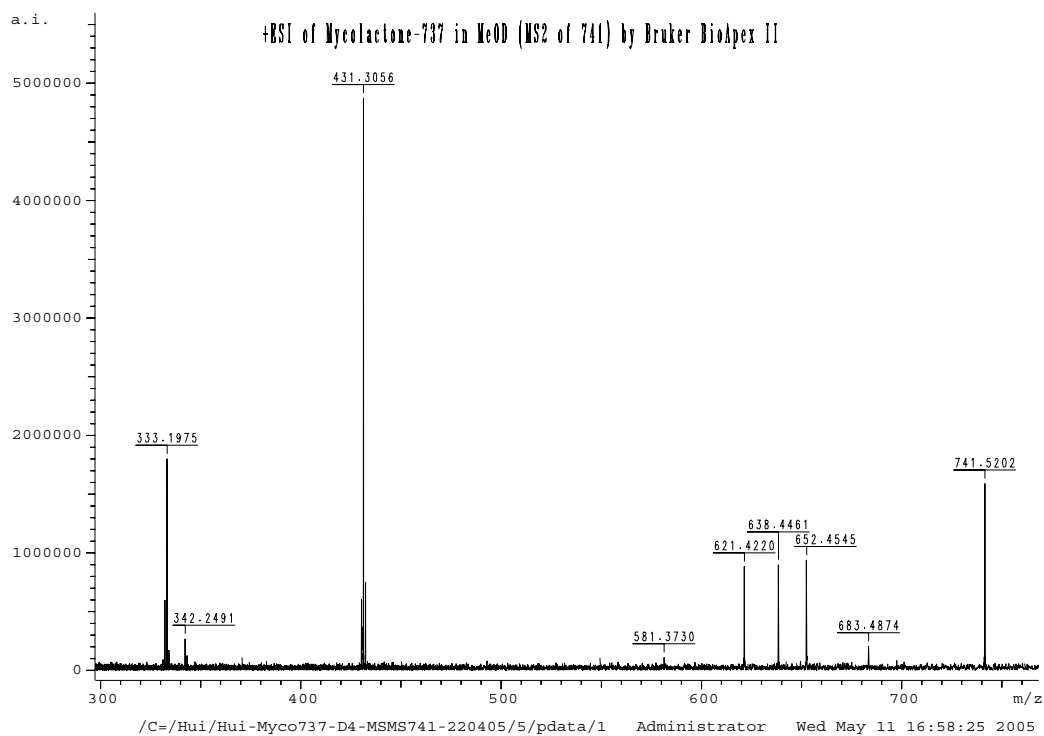
MUAgy99 <sup>a</sup> (African strain)				MU128FXT					
Metabolite [M+Na] <sup>+</sup>	Formula	DBE <sup>b</sup>	n <sup>c</sup>	Metabolite [M+Na] <sup>+</sup>	Formula	Observed mass	Error (ppm)	DBE <sup>b</sup>	n <sup>c</sup>
765	C <sub>44</sub> H <sub>70</sub> O <sub>9</sub> Na	10	5	737	C <sub>43</sub> H <sub>70</sub> O <sub>8</sub> Na	737.4980	-2.3	9	4
763	C <sub>44</sub> H <sub>68</sub> O <sub>9</sub> Na	11	4	735	C <sub>43</sub> H <sub>68</sub> O <sub>8</sub> Na	735.4821	-1.9	10	3

<sup>a</sup> The data for mycolactones from MUAgy99 are taken from reference [9]. <sup>b</sup> Double bond equivalent. <sup>c</sup> Number of deuterons after exchange.

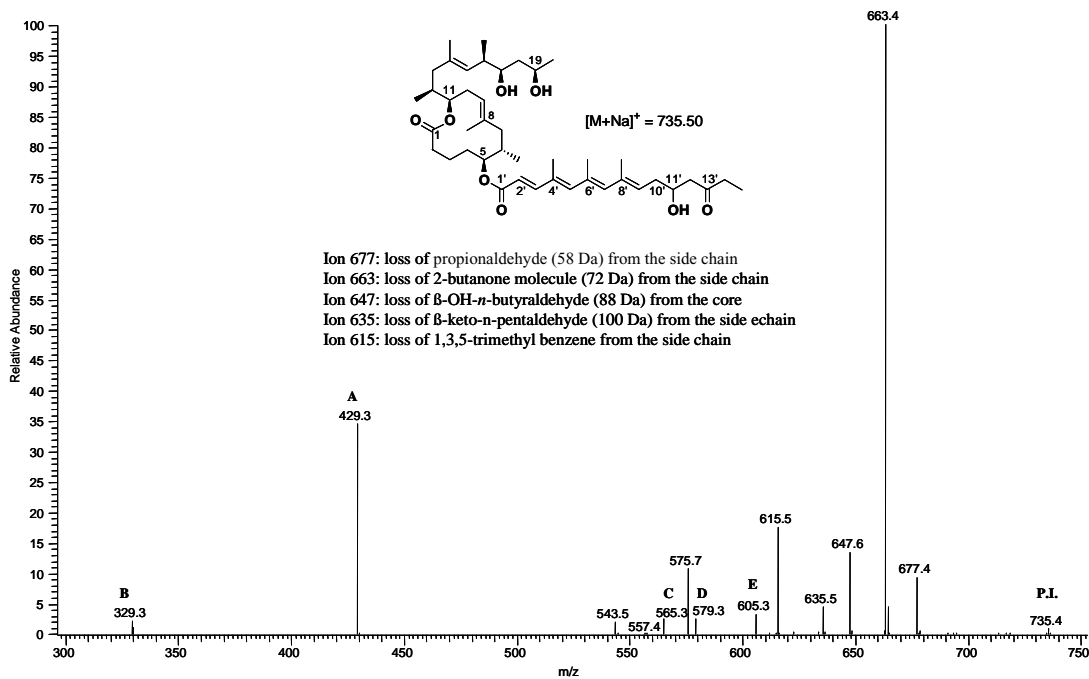
**Table 2** The formula, fragment ion identity, and observed mass for main ions observed in the high-resolution (FTICR) MS/MS experiment on mycolactone-737 from MU128FXT strain.

Formula	Fragment identity	Observed mass	Error (ppm)	Number of deuteriums
C <sub>43</sub> H <sub>70</sub> O <sub>8</sub> Na	Precursor ion	737.4942	2.8	4
C <sub>40</sub> H <sub>64</sub> O <sub>7</sub> Na	Loss of propionaldehyde from the side chain	679.4426	3.0	4
C <sub>39</sub> H <sub>62</sub> O <sub>6</sub> Na	Loss of β-OH- <i>n</i> -butyraldehyde from the core	649.4426	2.0	3
C <sub>38</sub> H <sub>60</sub> O <sub>6</sub> Na	Loss of β-OH- <i>n</i> -pentaldehyde from the side chain	635.4278	0.6	3
C <sub>34</sub> H <sub>58</sub> O <sub>8</sub> Na	Loss of 1,3,5-tri-methyl benzene from the side chain	617.3991	5.3	4
C <sub>36</sub> H <sub>54</sub> O <sub>6</sub> Na	Ion E <sup>a</sup>	605.3863	-8.2	2 <sup>b</sup>
C <sub>34</sub> H <sub>52</sub> O <sub>6</sub> Na	Ion D <sup>a</sup>	579.3657	-0.1	2
C <sub>33</sub> H <sub>50</sub> O <sub>6</sub> Na	Ion C <sup>a</sup>	565.3506	-1.2	2 <sup>b</sup>
C <sub>25</sub> H <sub>42</sub> O <sub>4</sub> Na	Ion A	429.2989	-3.2	2
C <sub>18</sub> H <sub>28</sub> O <sub>4</sub> Na	Ion B	331.1893	-4.0	2

<sup>a</sup> See Fig. 4. <sup>b</sup> From deuterated MS/MS spectrum from the LCQ.



## MS/MS spectrum of mycolactone-735 by Finnigan LCQ

MS<sup>3</sup> spectrum of 663 from MS/MS of 735 by Finnigan LCQ