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Note

Detection of tuberculostearic acid in mycobacteria and nocardiae by gas chromatography and mass spectrometry using selected ion monitoring

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Selected ion monitoring means the focusing of a mass spectrometer on fragments specific for the particular compound under study. When combined with gas chromatography (GC), the technique offers a very high degree of selectivity and sensitivity for the quantitative analysis of organic compounds which are volatile or can be converted into volatile derivatives. Many microorganisms produce such cellular or extracellular compounds, specific for a given genus or species [1, 2].

Tuberculosis is still one of the most important infectious diseases of mankind, the microbial diagnosis of which has in point of principle remained unchanged since the discovery of the causative organism. Thus, the isolation of *Mycobacterium tuberculosis* and several other pathogenic mycobacteria from clinical specimens takes several weeks. Mycobacteria, and also a limited number of other organisms of the order *Actinomycetales*, contain appreciable amounts of 10-methyloctadecanoic acid, mainly in the form of phospholipids in the cell wall. Because it is considered unique for these microorganisms, this acid is usually referred to as tuberculostearic acid [3—7].

In the present investigation we assessed whether selected ion monitoring of tuberculostearic acid could be used to detect mycobacteria, and thus, by

extension, be of use for the rapid diagnosis of mycobacterial infections.

Freshly isolated strains of *M. tuberculosis* were cultured in Proskauer—Beck medium, autoclaved, lyophilized and extracted with chloroform—methanol. The supernatants were then evaporated, methanolysed, and injected onto the GC column. By mass spectrometry (MS) it was demonstrated that one of the chromatographic peaks present in each chromatogram obtained represented the methyl ester of 10-methyloctadecanoic acid, the structure of which was confirmed by a comparative GC—MS study of the authentic compound, synthesized in our laboratory. The mass spectrum gives characteristic peaks, for instance at *m/e* 167 (loss of $CH_3(CH_2)_7$ followed by CH_3OH) and *m/e* 312 (molecular weight, M) (Fig. 1).

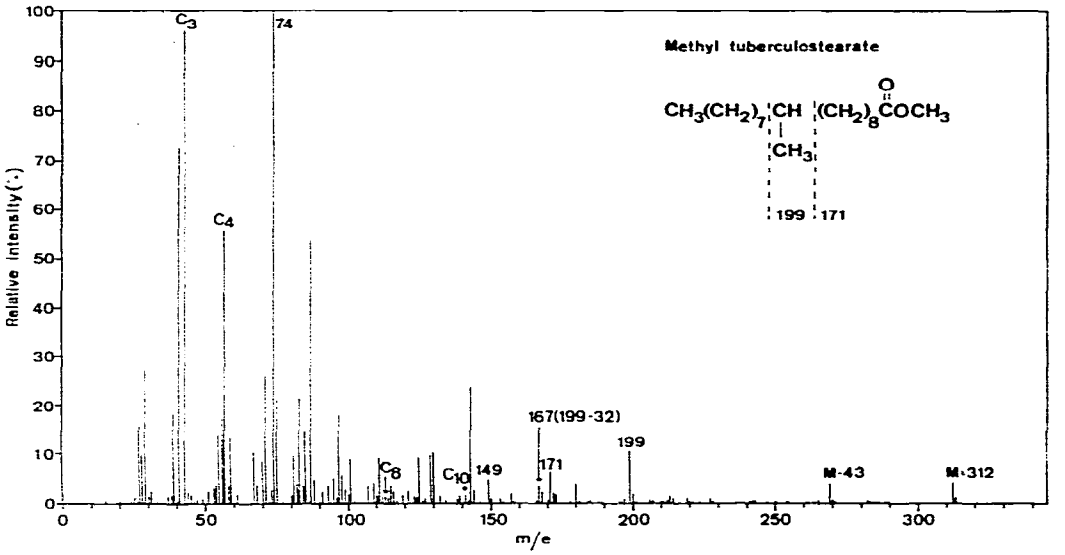

Fig. 1. Mass spectrum, in which characteristic fragments are indicated, of methyl tuberculostearate obtained from *Mycobacterium tuberculosis*. The bacteria had been cultured on Proskauer—Beck medium (Difco) at 37° for 30 days, autoclaved (121°, 60 min, 1.1 kP/cm²), washed with distilled water and lyophilized. Of the lyophilized cells, 2 mg were extracted with 1 ml of chloroform—methanol (2:1, v/v) overnight, after which the supernatant was evaporated to dryness under a stream of nitrogen, methanolysed for 20 h at 80° using 3% methanolic HCl, evaporated, and taken up in 100 µl of *n*-hexane. One microlitre was injected into a 2 m × 2 mm I.D. glass column packed with 3% OV-101 on Chromosorb W HP (80—100 mesh). The helium carrier gas flow-rate was 25 ml/min, and the column temperature 230°. A Varian MAT mass spectrometer was used, employing an ion source temperature of 230° and an electron energy of 70 eV.

The chromatograms obtained when analysing the extracts by monitoring at *m/e* 167 and *m/e* 312 contained one single peak, that of methyl 10-methyloctadecanoate. The greatest sensitivity was achieved at *m/e* 312, by which the fatty acid ester could be detected in as little as 2 ng of lyophilized cells, corresponding to 20 pg of ester. When employing ion selection at *m/e* 167, the signal-to-noise ratio was less favourable and the sensitivity decreased by a factor of approximately five.

M. tuberculosis, cultured on slants of Löwenstein—Jensen medium, was also

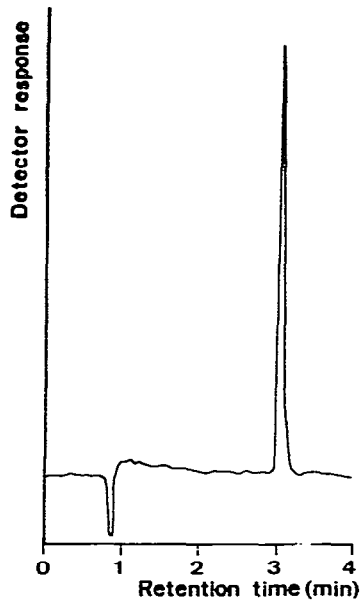

Fig. 2. Chromatogram from an extract of a culture of *Mycobacterium tuberculosis* obtained by selected ion monitoring at *m/e* 312. The bacteria had been collected by washing Löwenstein—Jensen slants with saline after five days of incubation at 37°. The preparation of the sample and the GC conditions were identical with those used in Fig. 1. Of a final solution of 100 µl of *n*-hexane, 0.1 µl was used for analysis. The peak represents approximately 200 pg of methyl tuberculostearate. The negative peak represents a pressure increase in the mass spectrometer caused by injection of the solvent.

tested. After 5 days of incubation at 37°, when still no colonies of mycobacteria could be detected by the naked eye, the slants were washed with saline and the suspensions so obtained treated as described above. A chromatogram using ion selection at *m/e* 312 is shown in Fig. 2. On duplicate cultures, mycobacterial colonies could first be visually observed 14—20 days after inoculation.

The specificity of the technique, when monitoring at *m/e* 167 and *m/e* 312, was evaluated in analyses of *M. africanum*, *M. avium*, *M. bovis* (Strain Bacillus Calmette-Guerin [BCG]), *M. kansasii*, *M. smegmatis*, *M. tuberculosis*, *Nocardia asteroides*, *N. brasiliensis* and *N. rubra*. In addition, *Escherichia coli* and strains of pneumococci, staphylococci, streptococci, and anaerobes, such as organisms of genera *Bacteroides*, *Clostridium* and *Propionibacterium*, were analysed. The chromatograms representing the mycobacteria and *Nocardia* sp. contained one peak, while those of the other bacteria studied were completely blank.

The GC—MS technique described provides chromatograms containing one single peak which are consequently easy to interpret. The sensitivity for tuberculostearic acid was found to be approximately 50 times greater than that obtainable with the flame ionization detector. Since many microbial products are unique for a particular genus or species, selected ion monitoring constitutes a diagnostic tool having great potential and wide scope for use within the field of clinical microbiology. The expense of mass spectrometry is a draw-back but should be related to the obvious advantages offered by the technique, such as a means for the rapid diagnosis of tuberculosis.

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