Journal of Chromatography, 190 (1980) 183–187

CHROM. 12,481

Note

Identification of characteristic branched-chain fatty acids of *Mycobacterium kansasii* and *gordonae* by gas chromatography–mass spectrometry

J. JULÁK

Laboratory for Special Medical Microbiology and Immunology, Charles University, Studničkova 7, 128 00 Prague 2 (Czechoslovakia)

F. TUREČEK

J. Heyrovský Institute of Physical Chemistry and Electrochemistry, Czechoslovak Academy of Sciences, Máchova 7, 121 38 Prague 2 (Czechoslovakia)

and

Z. MIKOVÁ

Laboratory for Special Medical Microbiology and Immunology, Charles University, Studničkova 7, 128 00 Prague 2 (Czechoslovakia)

(Received October 17th, 1979)

In previous papers concerning the composition of cellular fatty acids of *Mycobacterium kansasii*[1,2], we observed a characteristic fatty acid eluted by gas chromatography (GC) of methyl esters in the region between 14:0 and 15:0*. This acid, which formed 3–5% of cellular fatty acids, was described by Thoen *et al.*[3,4] as a characteristic component of *M. kansasii*, denoted as BCFA and characterized by mass spectrometry (MS) as a saturated branched-chain fatty acid with a methyl group at C-2.

In studies of the biochemical properties of scotochromogenic mycobacteria, namely *M. gordonae*, *flavescens* and *scrofulaceum*, we observed in lipids of *M. gordonae* the presence of an unidentified fatty acid. The retention of this component was identical with that of the Thoen *et al.*'s BCFA on an ethylene glycol adipate column, but slightly different on an OV-101 column. The relative abundance of the unknown acid corresponded to 2–8% of the total fatty acids. The mass spectrometry of both components showed the presence of a methyl group at C-2, but the presence of a further methyl group on C-4, C-6 or C-8 could not be excluded, especially in BCFA. An attempt to achieve a more precise characterization of these components of mycobacterial lipids is described in this paper.

EXPERIMENTAL

Seven strains of *M. gordonae*, five strains of *M. flavescens* and five strains of *M. scrofulaceum* were obtained from the collection of the Department of Tuberculosis, IHE, Prague, Czechoslovakia. The strains were cultivated for 8 weeks at 37° on Sauton's medium and on Šula's medium with enzymatic casein hydrolysate. The

* For abbreviations, see Table I.

sources and cultivation of *M. kansasii* were described earlier[1] (two type strains, 11 from patiens and 11 from water sources, grown on Lind's synthetic medium). From the inactivated dry biomass, freely bound lipids were extracted with chloroform–methanol (2:1) and converted into methyl esters with sodium methoxide in methanol and hydrochloric acid in methanol[5]. After extraction with light petroleum (b.p. 30–50°), the methyl esters of fatty acids up to C_{26} were analysed by GC on a Chrom 4 apparatus (Laboratorní přístroje, Prague, Czechoslovakia), equipped with a dual mass flow controler (Brooks, Hatfield, Pa., U.S.A.) and a flame-ionization detector, on a 1.2 m × 3 mm I.D. glass column packed with 3% OV-101 on Gas-Chrom Q (100–120 mesh) at an initial temperature of 180°, programmed after 25 min at 5°/min up to 250°, with a flow-rate of nitrogen of 10 ml/min, and on a 3.7 m × 3 mm I.D. stainless-steel column packed with 10% ethylene glycol adipate (EGA) on Chromosorb W AW (80–100 mesh) at 180° with a flow-rate of nitrogen of 20 ml/min.

Retention data for unknown mycobacterial and standard methyl esters were obtained also at 150° on both columns. The relative retentions (r_{is}) relative to methyl palmitate and methylmyristate of all components were calculated and from log r_{is} *versus* carbon number plots the equivalent chain lengths (ECLs) were determined. Their mean values, taken from $r_{i14:0}$ and $r_{i16:0}$ at 180° and 150°, were compared with those of standard methyl esters. The synthesis of standard methyl esters of 2-Me(14:0), 2,4-Me_2(13:0), 2,6-Me_2(13:0) and 2,8-Me_2(13:0) acids is described elsewhere[6]. GC–MS was performed on a Jeol JMS D-100 apparatus, ionizing energy 75 eV, on a 2 m × 3 mm I.D. column packed with 3.5% SE-30 on Chromosorb W at an initial temperature of 180°, programmed after 12 min at 5°/min up to 220°, with helium as carrier gas at 0.8 kp/cm² and with a two-step Becker-Ryhage separator maintained at 220°.

RESULTS AND DISCUSSION

Typical chromatograms of methyl esters from lipids of *M. flavescens* and *M. gordonae* are shown in Fig. 1. Strains of *M. flavescens* were found to have the fatty acid composition typical of most species of mycobacteria, with the presence of about 10% of tuberculostearic [19TB, *i.e.*, 10-Me(18:0)] acid and its lower homologue, 17TB. Strains of *M. scrofulaceum* showed similar fatty acid patterns, differing from those of *M. flavescens* in a lower content of monoenic acids. The fatty acid patterns of the seven strains of *M. gordonae* were unusual, differing from those of other mycobacteria by the complete absence or less than 0.5% of characteristic 19TB and 17TB acids and by the presence of about 2% of a component denoted as X in Fig. 1. The content of this component was higher (up to 8% of total fatty acids) when the same strains were grown on Šula's medium. It is of interest that the seven strains of *M. gordonae*, when investigated microscopically, were found to grow in cords, as did the strains of *M. kansasii*.

The ECLs for component X of *M. gordonae* were found to be 14.35 and 13.68 on OV-101 and EGA, respectively; an almost identical ECL of 13.89 was found for the component BCFA of *M. kansasii* on EGA, but its ECL was 14.63 on OV-101. The positions of both components were unchanged after catalytic hydrogenation and bromination of the samples. On comparing these values with the ECLs of standard branched-chain fatty acid methyl esters (Table I), we concluded that component X

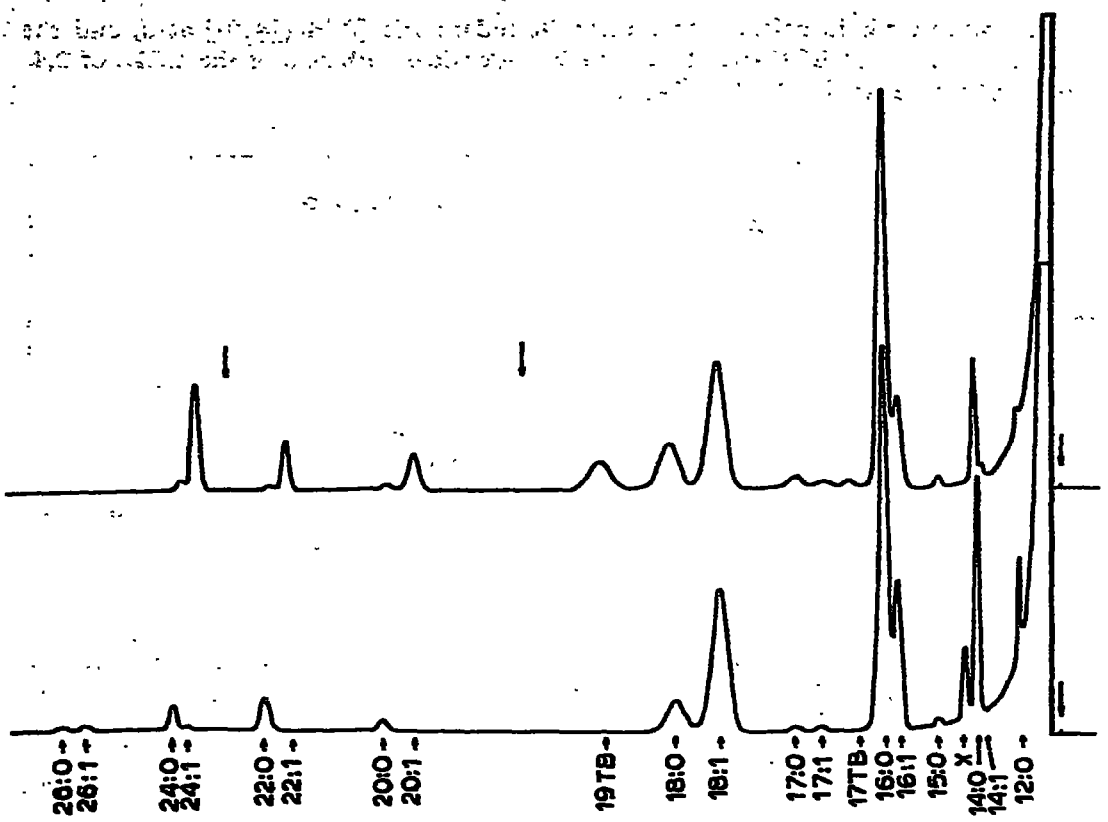

Fig. 1. Typical chromatograms of fatty acid methyl esters from *M. flavescens* strain 134/72 (upper trace) and *M. gordonae* strain 121/72 (lower trace) on OV-101. Start and end of temperature programming indicated by arrows.

TABLE I

ECL VALUES OF BRANCHED-CHAIN FATTY ACID METHYL ESTERS

Ester	*Column*	
	OV-101	*EGA*
2-Me (14:0)*	14.36	13.86
2,4-Me_2 (13:0)**	13.63 + 13.68 (13.66)	12.86 + 13.06
2,6-Me_2 (13:0)	13.74	13.11
2,8-Me_2 (13:0)	13.85	13.24
2,4-Me_2 (14:0)***	14.63 + 14.69 (14.66)	13.87 + 14.03

* Abbreviations according to Hansen[7]; *e.g.*, 2-Me (14:0) denotes a saturated fatty acid with 15 carbon atoms and a methyl group at C-2.

** A mixture of diastereoisomers, not resolved on OV-101 at 180° (values in parentheses).

*** Extrapolated from log r_{is} *versus* carbon number plot.

of *M. gordonae* is identical with 2-methyltetradecanoic [2-Me(14:0)] acid, and the ECL of component BCFA of *M. kansasii* is identical with one of the ECLs of 2,4-dimethyltetradecanoic [2,4-Me_2(14:0)] acid.

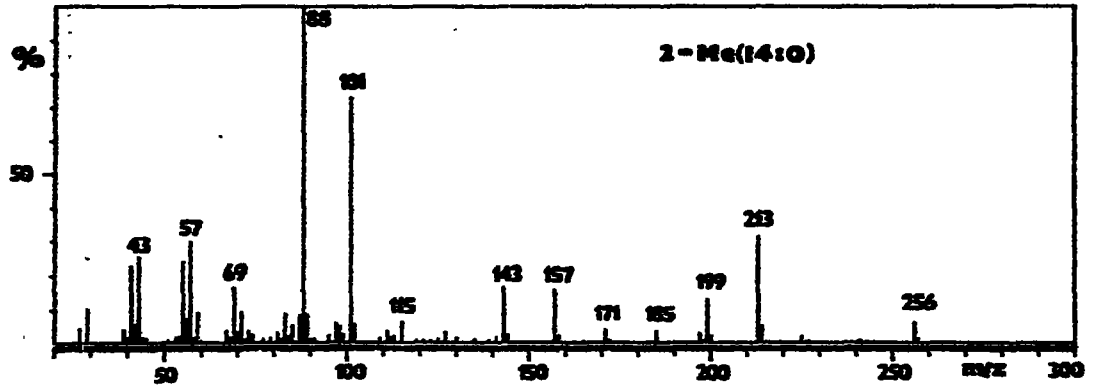

Fig. 2. Mass spectrum of 2-Me (14:0) methyl ester (authentic standard).

This identification was confirmed from the mass spectra of the compounds concerned. The mass spectrum of component X from *M. gordonae* was identical with that of authentic 2-Me(14:0) (Fig. 2), being characterized by presence of ions of *m/z* 88, 101, 143, 157, 199, 213 and 256. The mass spectrum of component BCFA from *M. kansasii* (Fig. 3) displays ions of *m/z* 88, 101, 129, 183 $(M-87)^+$, 211 $(M-59)^+$ and 270 $(M^{+\bullet})$, which confirm the location of methyl groups on C-2 and C-4 (ref. 8). The mass spectrum of the standard 2,4-Me_2(13:0)[6] (Fig. 4) shows a fragmentation pattern [ions of *m/z* 88, 101, 129, 169 $(M-87)^+$, 197 $(M-59)^+$ and 256 $(M^{+\bullet})$] very similar to that of BCFA. Hence, the presence of any further methyl groups in the latter acid, *e.g.*, in a position remote from the ester group, is highly improbable. It is noteworthing that diastereoisomers of 2,4-Me_2(13:0) can be separated by GC on SE-30[6], OV-101 and EGA. This implies that 2,4-Me_2(14:0) (component BCFA of *M. kansasii*), which gives a single peak, is present as a single diastereoisomer.

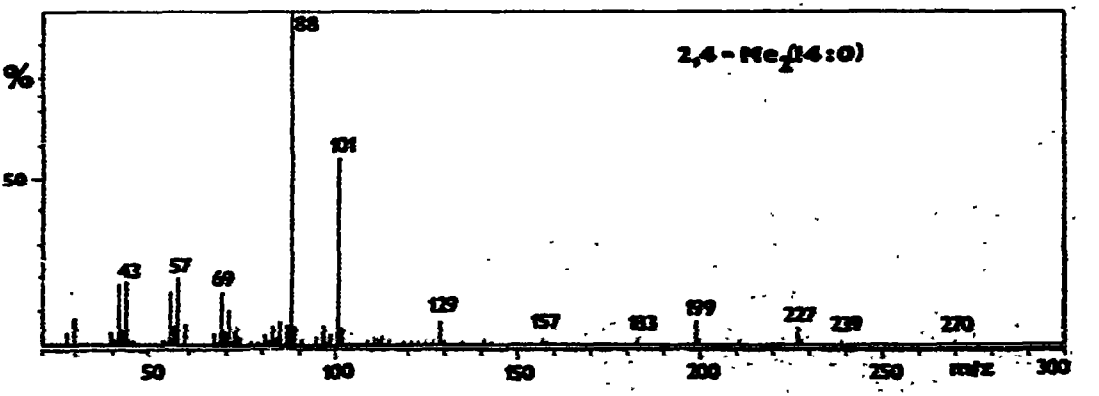

Fig. 3. Mass spectrum of 2,4-Me_2 (14:0) methyl ester (from lipids of *M. kansasii*).

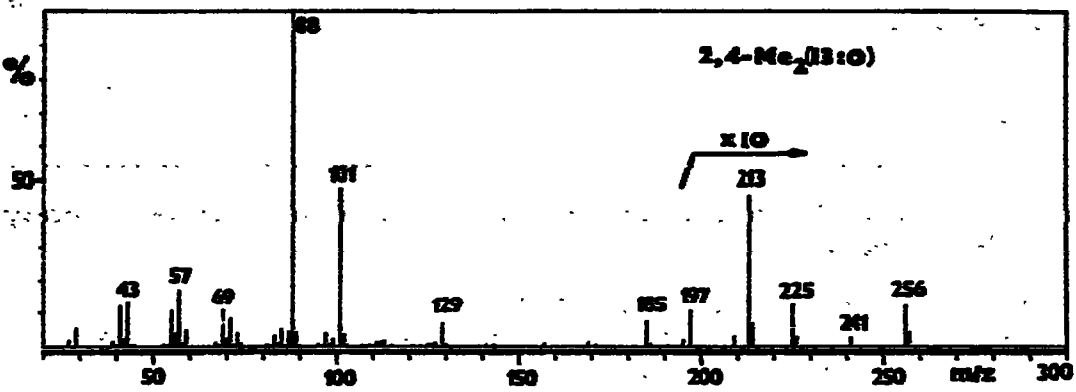

Fig. 4. Mass spectrum of 2,4-Me_2 (13:0) methyl ester (authentic standard).

Except for the mycolic acids with very long chains, we were unable to obtain any information in the literature about the occurrence of multiple branched-chain fatty acids in bacteria. They have been found in some waterfowl[9], fallow deer[10] and human skin[11]. The ECLs given in the last instance for 2-methyl and 2,6-dimethyl branched-chain fatty acids, although obtained on a different stationary phase, are in good agreement with our results. This also holds for the data concerning 2-methyl branched-chain acids given in refs. 12 and 13. The fatty acid patterns and, especially, the presence of 2-Me(14:0) fatty acid in *M. gordonae* may be of importance as a characteristic marker for the rapid diagnosis and the taxonomy of scotochromogenic mycobacteria instead of time-consuming and tedious biochemical tests.

REFERENCES

1 M. Kubín, M. Mára and J. Julák, *Cesk. Epidemiol. Mikrobiol. Imunol.*, in press.
2 J. Julák, M. Mára and M. Kubín, *Cesk. Epidemiol. Mikrobiol. Imunol.*, in press.
3 Ch. O. Thoen, A. G. Karlson and R. D. Ellefson, *Appl. Microbiol.*, 21 (1971) 628.
4 Ch. O. Thoen, A. G. Karlson and R. D. Ellefson, *Appl. Microbiol.*, 24 (1972) 1009.
5 R. L. Glass, *Lipids*, 6 (1971) 919.
6 F. Tureček, O. Turečková and J. Julák, *Collect. Czech. Chem. Commun.*, 44 (1979) 3111.
7 L. A. Hansen, *Lipids*, 6 (1971) 862.
8 H. Karlsson and G. Odham, *Ark. Kemi*, 31 (1969) 143.
9 A. K. Sen Gupta, *Fette, Seifen, Anstrichm.*, 74 (1972) 693.
10 A. Smith and W. R. H. Duncan, *Lipids*, 14 (1979) 350.
11 N. Nicolaides and J. M. B. Apon, *Biomed. Mass Spectrom.*, 4 (1977) 337.
12 T. A. Foglia, P. Heller and C. J. Dooley, *J. Amer. Oil Chem. Soc.*, 53 (1976) 45.
13 J. M. B. Apon and N. Nicolaides, *J. Chromatogr. Sci.*, 13 (1975) 467.