Journal of General Microbiology (1985), **131**, 2013–2021. *Printed in Great Britain*

Quantitative Comparison of the Mycolic and Fatty Acid Compositions of *Mycobacterium leprae* and *Mycobacterium gordonae*

By D. E. MINNIKIN,[1]* G. DOBSON,[1,2] M. GOODFELLOW,[2] P. DRAPER[3] AND M. MAGNUSSON[4]

[1]*Department of Organic Chemistry, The University, Newcastle upon Tyne NE1 7RU, UK*
[2]*Department of Microbiology, The University, Newcastle upon Tyne NE1 7RU, UK*
[3]*National Institute for Medical Research, Mill Hill, London NW7 1AA, UK*
[4]*Tuberculin Department, Statens Seruminstitut, Amager Boulevard 80, DK 2300 Copenhagen, Denmark*

(*Received 26 November 1984; revised 13 March 1985*)

The mycolic and fatty acids of three samples each of *Mycobacterium leprae* and *Mycobacterium gordonae* were compared. Acids released by whole-organism alkaline hydrolysis were converted to 4-nitrobenzyl esters and mycolic acids were further derivatized to *t*-butyldimethylsilyl ethers. Thin-layer chromatography of the derivatized long-chain extracts showed that all three *M. leprae* preparations contained so-called α-mycolates and ketomycolates but that the *M. gordonae* samples had a methoxymycolate in addition to the above types. Silica gel normal-phase high-performance liquid chromatography of the total mycolic acid derivatives confirmed the lack of detectable amounts of methoxymycolates in *M. leprae* and reverse-phase chromatography of the individual mycolate types demonstrated the homogeneity of the chain lengths of the mycolic acids in each species. Non-hydroxylated fatty acid 4-nitrobenzyl esters were transformed to methyl esters and examined by gas chromatography. Tuberculostearic (10-methyloctadecanoic) acid was a major component of the lipids of all three *M. leprae* preparations but it was absent in one *M. gordonae* strain and a very minor component in the other representatives of this latter species. On the basis of fatty and mycolic acid compositions, therefore, a previously suggested close relationship between *M. leprae* and *M. gordonae* was not supported.

INTRODUCTION

An experimental vaccine, consisting of killed whole *Mycobacterium leprae*, has given good results in animal tests (see, for example, Shepard *et al.*, 1980, 1983) and in immunotherapy trials (Convit *et al.*, 1982), and it is beginning to be tested as a prophylactic against human leprosy. The bacteria are obtained from tissues of experimentally infected nine-banded armadillos inoculated with bacteria originally derived from human biopsies.

Since *M. leprae* has apparently not been cultivated on laboratory media, the types of test that can be used to confirm its identity are restricted and have depended on immunological procedures and chemical criteria such as lipid composition. It has caused some concern that there appeared to be a discrepancy between the lipid compositions of *M. leprae* determined in different laboratories. The differences concerned the mycolic acid composition and absence of tuberculostearic acid; in both these characters *M. leprae*, according to Asselineau *et al.* (1981) and Daffé *et al.* (1981), resembled the cultivable species *M. gordonae*. Both organisms were considered to produce α-mycolic acids (Fig. 1, I), ketomycolic acids (Fig. 1, II) and methoxymycolic acids (Fig. 1, III) and to lack tuberculostearic (10-methyloctadecanoic) acid.

Abbreviation: TBDMS, *t*-butyldimethylsilyl.

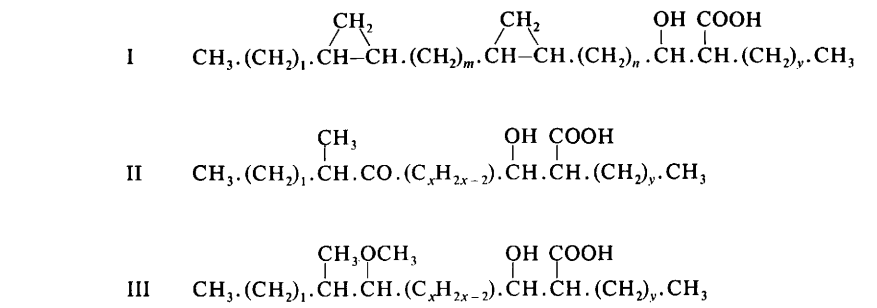

Fig. 1. General structures of mycolic acids.

This conflicted with an original report by Etémadi & Convit (1974) that *M. leprae* from human biopsies contains only α- and ketomycolates.

This paper describes a detailed analysis of mycolic and fatty acid compositions of three batches of *M. leprae* purified from tissues of different armadillos inoculated from different sources, and also of three authentic strains of *M. gordonae*.

METHODS

Strains and cultivation. Three 5 mg samples of *Mycobacterium leprae* were purified from separate armadillo livers (W39, W77 and L424), as described previously (World Health Organization, 1980). Armadillo W39 had been inoculated with bacteria from human tissue; W77, however, had been inoculated with bacteria from another armadillo experimentally infected from the same human source as W39. Armadillo L424 had been infected with bacteria from a separate human source. Three *Mycobacterium gordonae* strains, MNC 64, 661 and 662, were grown as described previously (Minnikin *et al.*, 1984*b*). Freeze-dried heat-killed *Mycobacterium avium* D4 and *Mycobacterium tuberculosis* C were supplied by H. B. Lee, Central Veterinary Laboratory, Weybridge, UK.

Extraction and derivatization of fatty and mycolic acids. Freeze-dried preparations (5–6 mg) of the three *M. leprae* samples and 50 mg of each of the other strains were hydrolysed with a mixture of methanol (1 ml), 30% aq. KOH (1 ml) and toluene (0·1 ml) in a tube sealed with a polytetrafluoroethene-lined screw cap at 75 °C for 3 d. Acidification with concentrated hydrochloric acid was followed by extraction with toluene (3 × 1 ml) and the extract was evaporated to dryness. A phase transfer catalysis method (Dobson *et al.*, 1985) was used for the preparation of 4-nitrobenzyl esters. Samples of hydrolysates were dissolved in dichloromethane (1 ml) in a closed tube to which aqueous solutions of 0·1 M-tetrabutylammonium hydrogen sulphate plus 0·2 M-NaOH (1 ml) and 4-nitrobenzyl bromide (10 mg for *M. leprae* samples, 25 mg for others) were added. These were mixed for 30 min, the layers were allowed to settle, the upper aqueous layer was discarded and the lower layer was evaporated to dryness and left overnight in a desiccator with concentrated H_2SO_4.

Fatty and mycolic acid methyl esters of the three *M. gordonae* strains were extracted by alkaline methanolysis (Minnikin *et al.*, 1982, 1984*a*). Freeze-dried cells (50 mg) were left overnight at 37 °C in a mixture of methanol (1 ml), toluene (0·5 ml) and tetramethylammonium hydroxide (0·5 ml) in a sealed tube, centrifuged and the supernatant removed. The residue was washed with methanol/toluene (2:1, v/v, 1 ml) and the combined supernatants were shaken with a 2·5% mixture of iodomethane in *N,N*-dimethylformamide (4 ml) for 1 h, followed by three extractions with petroleum ether (b.p. 60–80 °C; 1 × 4 ml, 2 × 2 ml). The combined petroleum ether extracts were evaporated to less than 1 ml under nitrogen at 37 °C, extracted twice with petroleum ether (b.p. 60–80 °C; 2 × 0·5 ml), and the extracts evaporated to dryness under nitrogen. Fatty acid 4-nitrobenzyl esters were converted to methyl esters by acid methanolysis (Minnikin *et al.*, 1980). *t*-Butyldimethylsilyl (TBDMS) ethers (Dobson *et al.*, 1985) of 4-nitrobenzyl and of methyl esters were prepared by treating a solution in toluene (0·15 ml) with TBDMS reagent (0·15 ml), prepared by dissolving 0·15 g *t*-butyldimethylsilyl chloride and 0·17 g imidazole in 1 ml dimethylformamide. The solution was left at 75 °C overnight, cooled, and extracted with petroleum ether (b.p. 60–80 °C; 3 × 0·3 ml). The extract was passed through a 1 cm column of aluminium oxide prewashed with diethyl ether, the column was washed with diethyl ether (2 × 0·5 ml) and the combined extracts were evaporated to dryness.

Analytical and preparative thin-layer chromatography (TLC). Analytical TLC was done on 6·6 × 6·6 cm plates cut from Merck 5554 silica gel $60F_{254}$ aluminium sheets. Two-dimensional (2-D) TLC, with a triple development with petroleum ether (b.p. 60–80 °C)/ethyl acetate (98:2, v/v) in the first direction followed by a single development in the second direction with petroleum ether (b.p. 60–80 °C)/acetone (98:2, v/v) (Minnikin *et al.*, 1983*a*), was used for the TBDMS ethers of 4-nitrobenzyl esters of the total fatty acids in all strains. Additionally, 2-D TLC was done

on samples of *M. leprae* and *M. tuberculosis* using single developments of toluene and petroleum ether (b.p. 60–80 °C)/acetone (98:2, v/v) in the first and second directions, respectively. One-dimensional (1-D) TLC of the TBDMS ethers of mycolic 4-nitrobenzyl and methyl esters was done with petroleum ether (b.p. 60–80 °C)/toluene (50:50, v/v and 60:40, v/v, respectively); for the fatty acid methyl esters of all strains, toluene/acetone (99:1, v/v) was used.

Preparative 2-D TLC was done, to purify the fatty acid 4-nitrobenzyl esters from the TBDMS ethers of the total mycolic acid 4-nitrobenzyl esters in the strains of *M. leprae* and *M. tuberculosis*, on 10 × 10 cm pieces of Merck 5735 silica gel $60F_{254}$ plastic sheets with toluene in the first direction and petroleum ether (b.p. 60–80 °C)/acetone (92:2, v/v) in the second direction. Similar purifications were done for all other strains on 10 × 10 cm plastic sheets in one dimension using petroleum ether (b.p. 60–80 °C)/acetone (96:4, v/v). The TBDMS ethers of the mycolic acid 4-nitrobenzyl and methyl esters were purified by 1-D TLC on plastic-backed plates in petroleum ether (b.p. 60–80 °C)/toluene (50:50, v/v, and 60:40, v/v, respectively).

Components on analytical TLC plates were revealed by spraying with a 5% ethanolic solution of molybdophosphoric acid followed by heating at 150 °C for 15 min. Components on preparative TLC plates were revealed under UV light either directly or after spraying with a 0·01% ethanolic solution of Rhodamine 6G.

Instrumentation. Mass spectra of TBDMS ethers of mycolic acid methyl esters from *M. gordonae* strains were taken on an AEI MS9 instrument with a direct insertion probe, an ionizing voltage of 70 eV and a temperature range of 240 to 270 °C.

Gas chromatography (GC) of fatty acid methyl esters from the strains of *M. gordonae* and *M. leprae* was done on a Perkin–Elmer F11 instrument with a column (1 m × 2 mm i.d.) packed with 3% OV-1 on 80–100 mesh Gas Chrom Q. The temperature was held at 170 °C for 15 min and then programmed from 170 to 250 °C at a rate of 2 °C min^{-1}. Isothermal analyses were done at 210 °C using columns (6 m × 2 mm i.d.) packed with 3% OV-1 on 80–100 mesh Gas Chrom Q or 10% Silar 10C on 100–120 mesh Gas Chrom Q.

High-performance liquid chromatography (HPLC) of TBDMS ethers of mycolic acid 4-nitrobenzyl esters was done with a Perkin–Elmer Series 2 pump, a Waters RCM-100 radial compression module and a Perkin–Elmer LC-55 ultraviolet detector (257 nm). The total derivatized mycolate samples were run on a silica gel Waters Radial-pak B cartridge and separated mycolate types, purified by TLC or by HPLC for the *M. leprae* samples, were run on a reverse-phase Waters Radial-pak A cartridge. Other conditions are given with the appropriate figures. Copies of the complete TLC and HPLC data have been deposited with the British Library Lending Division, Boston Spa, Yorkshire LS23 7BQ, UK, as Supplementary Publication no. SUP 28019 (5 pp.). (Copies may be obtained from the BLLD on demand; wherever possible, requests should be accompanied by prepaid coupons, held by many university and technical libraries and by the British Council.)

RESULTS

TLC patterns of 4-nitrobenzyl esters of TBDMS derivatives of mycolic acids of *M. leprae* and *M. gordonae* are shown in Fig. 2, and compared with patterns of mycolic acid derivatives of standard *M. tuberculosis* and *M. avium*. All the TLC patterns contained components corresponding to 4-nitrobenzyl esters of non-hydroxylated fatty acids and TBDMS ethers of 4-nitrobenzyl esters of α- and ketomycolic acids. As expected, the pattern from *M. avium* also contained a component corresponding to a derivative of an ω-carboxymycolic acid while a methoxymycolic acid derivative was seen in the *M. tuberculosis* and in all three *M. gordonae* patterns. The patterns from all three *M. leprae* samples did not contain spots corresponding to methoxy or ω-carboxymycolate derivatives (Fig. 2) but three additional components (P_1–P_3), which did not absorb UV light, were observed. These latter three lipids were also seen in the pattern from *M. tuberculosis* (Fig. 2) and correspond to dimycocerosates of phthiocerol A, phthiocerol B and phthiodiolone A as identified previously (Minnikin *et al.*, 1983*a*).

The TBDMS ethers of the 4-nitrobenzyl esters of the mycolic acids from the strains of *M. gordonae* and *M. leprae*, isolated together by preparative TLC, were analysed by silica gel HPLC and representative results are shown in Fig. 3. Mycolic acid types were identified by comparison with the retention times of derivatives of α-, keto- and methoxymycolic acids from *M. tuberculosis*. Samples of the individual mycolates were collected and analysed by reverse-phase HPLC as shown for the same representatives in Fig. 4. The numbers of carbons in the separated components (Fig. 4) were assigned by co-chromatography with derivatives of mycolic acids of known size from *M. avium* and *M. tuberculosis*.

The proportions of the α-mycolates and ketomycolates for *M. leprae* samples W77 and L424 were 78·6 and 21·4% and 77·6 and 22·4%, respectively, both having a close similarity to the

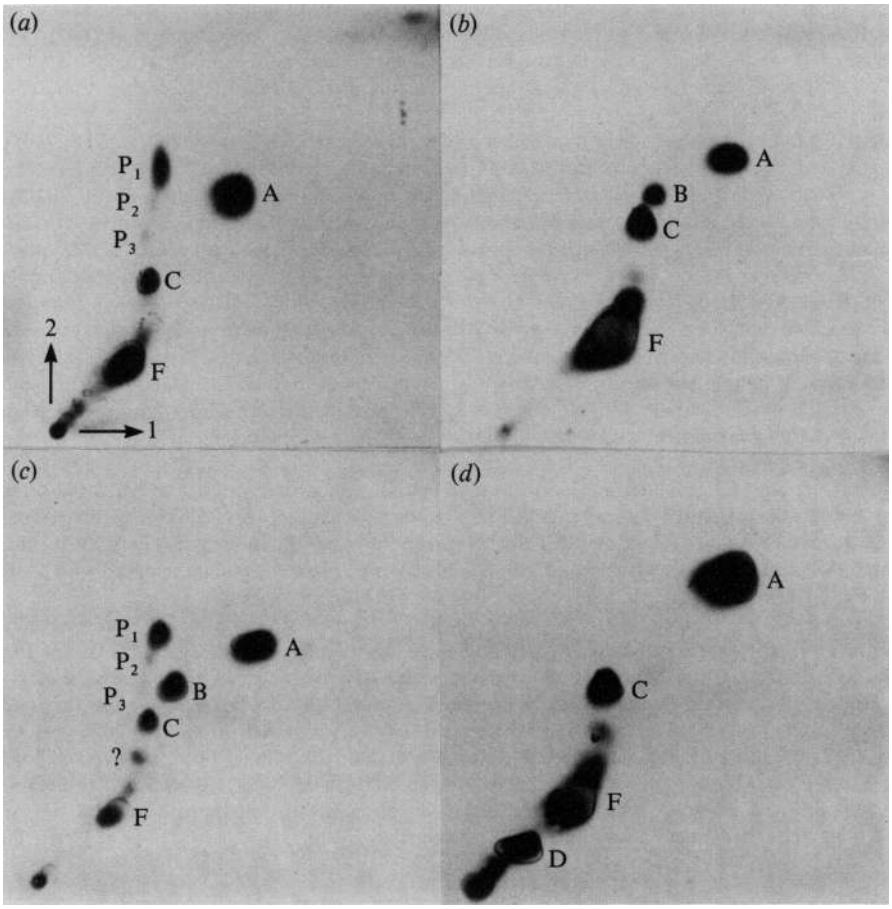

Fig. 2. TLC analysis of TBDMS ethers of 4-nitrobenzyl esters of whole-organism alkaline hydrolysates of (*a*) *M. leprae* W39, (*b*) *M. gordonae* MNC 64, (*c*) *M. tuberculosis* C and (*d*) *M. avium* D4. A triple development with petroleum ether (b.p. 60–80 °C)/ethyl acetate (98:2, v/v) in the first direction was followed by a single development with petroleum ether (b.p. 60–80 °C)/acetone (98:2, v/v) in the second direction. F, non-hydroxylated fatty acid 4-nitrobenzyl esters; A–C, TBDMS ethers of 4-nitrobenzyl esters of α-mycolates, methoxymycolates and ketomycolates; D, TBDMS ether of 4-nitrobenzyl diesters of ω-carboxymycolates; P_1–P_3, dimycocerosates of phthiocerol A, phthiocerol B and phthiodiolone A; ?, unknown.

proportions shown for *M. leprae* W39 in Fig. 3. The *M. gordonae* strains MNC 661 and 662 also gave profiles on silica gel HPLC which were quantitatively close to that for strain MNC 64 (Fig. 3), the ratios for α-, methoxy- and ketomycolates being 41·4, 11·2 and 47·4% and 40·6, 12·5 and 46·9%, respectively, for strains MNC 661 and 662. The profiles of the homologous components of the α- and ketomycolates from *M. leprae* W77 were very similar to those for W39 (Fig. 4) but minor relative increases were seen for the C_{80} α-mycolates and C_{85} ketomycolates of *M. leprae* L424. The reverse-phase HPLC profiles of the α-, methoxy- and ketomycolates from *M. gordonae* MNC 661 and 662 were essentially the same as that for strain MNC 64 (Fig. 4) with minor quantitative variations.

The overall sizes of the mycolic acids from the three strains of *M. gordonae* were also determined by mass spectrometry of the TBDMS ethers of mycolic acid methyl esters. These mycolic acid derivatives fragment, on mass spectroscopy, to give intense M-57 ions due to the loss of a *t*-butyl group (Minnikin *et al.*, 1981). For the α-mycolate derivatives of the strains of *M. gordonae* the principal M-57 fragments were at *m/z* 1179, 1207 and 1235, corresponding to C_{76}, C_{78} and C_{80} parent mycolic acids. The methoxymycolate derivatives all gave major M-57–32

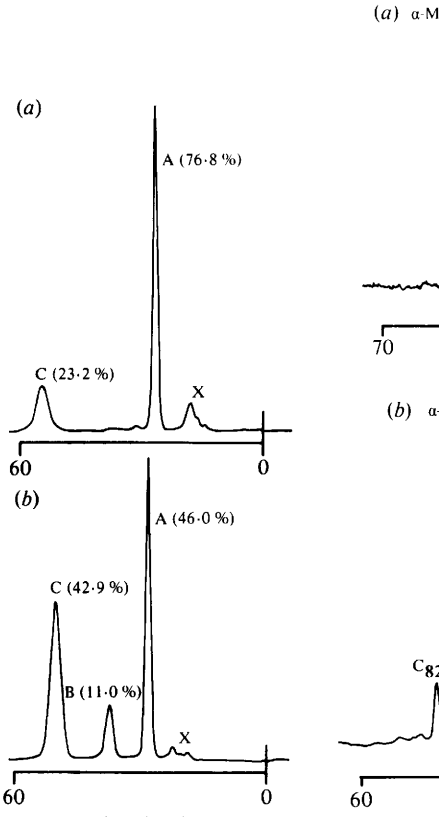

Fig. 3

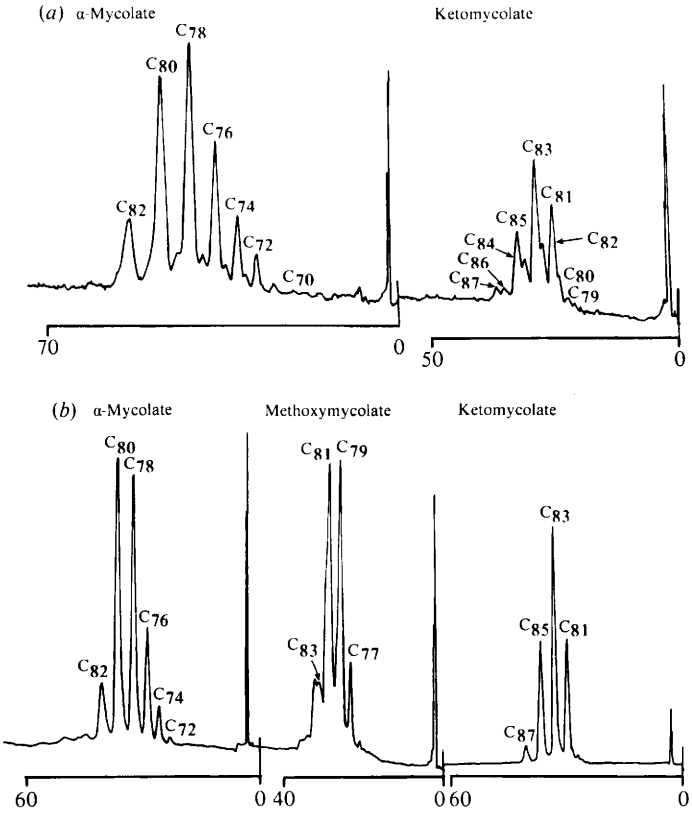

Fig. 4

Fig. 3. Adsorption HPLC of TBMS ethers of 4-nitrobenzyl esters of the total mycolic acids from (*a*) *M. leprae* W39 and (*b*) *M. gordonae* MNC 64. A Waters Radial-pak B cartridge was used with hexane/tetrahydrofuran (99:1, v/v) at a flow rate of 0·3 ml min^{-1}. A–C, TBDMS ethers of 4-nitrobenzyl esters of α-mycolates, methoxymycolates and ketomycolates; X, non-mycolic acid material eluting with the solvent front. Proportions of components are shown in parentheses.

Fig. 4. Reverse-phase HPLC of TBDMS ethers of 4-nitrobenzyl esters of mycolic acids from (*a*) *M. leprae* W39 and (*b*) *M. gordonae* MNC 64. A Waters Radial-pak A cartridge was used with acetonitrile/tetrahydrofuran (1:1, v/v) at 1·5 ml min^{-1} for α-mycolates and methoxymycolates and 1·0 ml min^{-1} for ketomycolates. Separated components are labelled with the number of carbons in the parent underivatized mycolic acid.

peaks at *m/z* 1207 and 1235, corresponding to parent C_{79} and C_{81} mycolic acids; *M. gordonae* MNC 662 also had a major C_{77} component (*m/z* 1179). All the ketomycolate derivatives had M-57 peaks at *m/z* 1265 and 1293 for C_{81} and C_{83} parent mycolic acids and strain MNC 661 also had a major C_{85} acid (*m/z* 1321). The total size ranges of the α-, methoxy-, and ketomycolates were estimated by mass spectrometry to be C_{72}–C_{84}, C_{75}–C_{86} and C_{80}–C_{88}, respectively. All of the TBDMS ether derivatives of the *M. gordonae* mycolates had a major peak at *m/z* 467, accompanied by a minor peak at *m/z* 439. These peaks are fragments arising by cleavage between the third and fourth mycolic acid carbon atoms and loss of 58 mass units and correspond to C_{22} and C_{20} chains in 2-positions.

Non-hydroxylated fatty acid methyl esters from *M. leprae* and *M. gordonae* were analysed by GC and the quantitative results are given in Table 1. The major components were straight-chain, unsaturated and 10-methyloctadecanoic (tuberculostearic) acids which were identified by standards and the calculation of equivalent chain lengths. The proportions of tuberculostearic acid in the strains of *M. gordonae* were particularly low when compared with those from *M. leprae*.

Table 1. *Percentage fatty acid composition of M. gordonae and M. leprae*

Retention time (min)* ...	1·9	3·4	4·1	4·6	7·2	7·7	10·0	11·2	21·8	23·9	26·0	32·0	33·6	36·0	40·3	41·5	46·0	47·6	48·5	51·0
ECL(OV-1,6m)† ...	12·0	13·6	13·8	14·0	15·0	15·3	15·8	16·0	17·8	18·0	18·4									
Assignment‡ ...	12:0	A	14:1	14:0	15:0	B	16:1	16:0	18:1	18:0	Me18	C	20:0	D	22:1	22:0	E	F	24:0	G
M. gordonae																				
MNC 64	0·1	tr	tr	7·8	tr	–	5·7	41·6	23·5	9·8	–	2·7	3·3	tr	0·1	2·9	–	tr	2·5	tr
MNC 661	tr	0·3	0·4	4·0	tr	tr	6·9	32·7	36·8	5·6	1·2	2·1	1·7	0·5	1·7	1·6	–	1·4	2·2	0·7
MNC 662	tr	0·2	–	2·3	tr	tr	4·1	37·9	31·3	8·2	2·3	tr	2·2	1·1	tr	4·3	0·2	–	3·6	2·3
M. leprae																				
W39	tr	tr	tr	0·7	0·2	tr	1·5	32·7	34·8	11·1	12·7	0·5	1·4	0·7	tr	2·1	0·1	tr	0·3	1·2
W77	–	0·1	tr	0·5	tr	2·1	1·6	26·9	34·1	10·1	12·4	2·9	2·1	0·7	0·9	2·4	0·9	tr	0·4	1·6
L424	–	tr	0·2	0·6	tr	tr	1·5	32·1	30·3	8·0	17·7	0·4	0·9	1·3	1·2	1·9	0·5	0·7	0·7	1·9

tr, Trace.

* Percentages and retention times measured using a 1 m, OV-1 column with an initial 5 min at 170 °C followed by temperature programming at 2 °C min^{-1} to 250 °C.

† Equivalent chain lengths (Miwa *et al.*, 1960) calculated using a 6 m, OV-1 column, isothermally at 220 °C. On a 6 m Silar 10C column all profiles showed the presence of an additional minor component with an ECL of 18·5, compared with an ECL of 18·7 for 18:1.

‡ Fatty acid abbreviations exemplified by: 16:0, hexadecanoic acid; 16:1, hexadecenoic acid; Me18, 10-methyloctadecanoic (tuberculostearic) acid.

A–G, unidentified components.

DISCUSSION

Mycobacterium leprae obtained from human biopsy specimens (Etémadi & Convit, 1974) contained α-mycolic acids (Fig. 1, I) and ketomycolic acids (Fig. 1, II); chromatographically similar acids were found in armadillo-grown bacteria by Draper (1976). The same simple pattern of α- and ketomycolic acids was found in both armadillo-grown and biopsy-derived bacteria by Young (1980). However, in studies based on mass spectroscopy, Asselineau *et al.* (1981) found evidence for a third type (methoxymycolate; Fig. 1, III) in a single specimen of armadillo-derived *M. leprae*. Two additional detailed investigations found only two types in armadillo-grown *M. leprae*: α- and ketomycolates (Draper *et al.*, 1982); and α-mycolates and an unspecified oxygenated mycolate (Kusaka *et al.*, 1982).

In the present study the mycolic acid compositions of three separate preparations of *M. leprae* were closely similar to each other (Figs 2–4). The only mycolic acids detected were major amounts of α-mycolic acids and lesser proportions of ketomycolic acids (Figs 2 and 3); methoxymycolates were not observed. Representatives of *M. gordonae*, however, contained similar proportions of α-mycolates and ketomycolates with lesser amounts of methoxymycolates (Figs 2 and 3). The profiles recorded by reverse-phase HPLC (Fig. 4) for the mycolates of *M. leprae* showed that the size ranges were also very similar to each other as were those for the mycolates of *M. gordonae*. The size ranges of the latter mycolates were checked by mass spectrometry. The α-mycolates from *M. leprae* had a major series, apparently corresponding to acids with even numbers of carbon atoms, having two *cis*-cyclopropane rings as characterized previously (Draper *et al.*, 1982); small amounts of a minor series with odd numbers of carbons were also observed (Fig. 4). The major and minor series of ketomycolates from *M. leprae* (Fig. 4) had odd and even numbers of carbon atoms corresponding to acids with *trans*- or *cis*-cyclopropane rings, respectively (Draper *et al.*, 1982). In contrast, the ketomycolates and α-mycolates of *M. gordonae* appeared to consist of a single series of homologous acids (Fig. 4) whose precise structures remain to be determined. The methoxymycolates from *M. gordonae*, however, appeared to consist of two overlapping homologous series (Fig. 4). The present results are in good agreement with a previous report on the composition of the mycolic acids of *M. leprae* harvested from experimentally-infected armadillos (Draper *et al.*, 1982), but they do not support the suggestion that *M. leprae* from the same source produces a methoxymycolic acid (Asselineau *et al.*, 1981). The detection of methoxymycolates in all three strains of *M. gordonae*, in addition to α- and ketomycolates, supports the previous reports of Daffé *et al.* (1981) and Minnikin *et al.* (1984*b*) and distinguishes this species from *M. leprae*. It should be noted that the TLC patterns of the mycolic acid derivatives from *M. leprae* (Fig. 2) all include components corresponding to dimycocerosates of the phthiocerol family. These characteristic waxes are relatively resistant to hydrolysis (Draper *et al.*, 1983; Minnikin *et al.*, 1983*a*). In previous studies (Draper *et al.*, 1983; Hunter & Brennan, 1983) dimycocerosates of phthiocerol A from *M. leprae* were analysed; dimycocerosates of the minor phthiocerol B and phthiodiolone A components were detected in *M. leprae* (Fig. 2) for the first time in the present study.

The non-hydroxylated fatty acid composition of armadillo-grown *M. leprae* has been examined by several groups. Asselineau *et al.* (1981) were unable to find 10-methyloctadecanoic acid (tuberculostearic acid) in a single sample studied of *M. leprae*. This absence is unusual in a species of *Mycobacterium*, but is seen also in *M. gordonae* (Daffé *et al.*, 1981), and, on the basis of this feature and the apparent presence of methoxymycolate in *M. leprae*, it was proposed that these two species were closely related. On the other hand, Andersen *et al.* (1982) and Kusaka & Izumi (1983) found that *M. leprae* had fatty acid patterns typical of mycobacteria, with straight-chain, unsaturated and tuberculostearic acids. The patterns found in the three batches of *M. leprae* used in the present work, were very similar to each other and to that recorded by Andersen *et al.* (1982). Tuberculostearic acid was always present in substantial proportions. By the addition of the unnatural tridecanoic acid as an internal standard it has been shown that the total fatty acid concentrations of preparations W39, W77 and L424 were 9·1, 8·2 and 9·4%, respectively (P. Draper & S. N. Payne, unpublished results), indicating that the preparations are similar in respect of fatty acid content.

The strains of *M. gordonae* either lacked tuberculostearic acid or possessed it in very minor amounts, confirming the work of others (Tisdall *et al.*, 1979; Julák *et al.*, 1980; Daffé *et al.*, 1981; Alvin *et al.*, 1983). Some groups have detected small amounts of 2-methyltetradecanoic acid in *M. gordonae* (Tisdall *et al.*, 1979; Julák *et al.*, 1980). The latter group quote an equivalent chain length for this compound of 14·36, using a column packed with OV-101, which is similar to the OV-1 phase used in the present experiments. As may be seen from Table 1, no similar component was seen in the fatty acid profiles of *M. gordonae* recorded here.

The relatively simple pattern of α- and ketomycolates found in *M. leprae* is quite unusual, occurring elsewhere only in some substrains of *M. bovis* BCG (Minnikin *et al.*, 1983*b*, 1984*c*; Daffé *et al.*, 1983). The 20- and 22-carbon side-chains of the mycolates are, however, different from the 24-carbon side-chains of mycolates of *M. bovis* BCG. As discussed in the accompanying paper (Minnikin *et al.*, 1985), evidence from other complex lipids also indicates a relationship between *M. bovis* (among others) and *M. leprae*. There seems no basis for supposing a close relationship to *M. gordonae* as suggested by Asselineau *et al.* (1981) and Daffé *et al.* (1981). The *M. leprae* used by these workers was from a similar source to those in the present study but the method of preparation and analytical procedures were quite different.

G. D. was supported by a studentship from the British Leprosy Relief Association (LEPRA) and the work was aided by a grant from the IMMLEP Steering Committee of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (T16/181/L4/29). Mass spectra were recorded by S. H. Addison and P. Kelly.

REFERENCES

ALVIN, C., LARSSON, L., MAGNUSSON, M., MÅRDH, P.-A., ODHAM, G. & WESTERDAHL, G. (1983). Determination of fatty acids and carbohydrate monomers in micro-organisms by means of glass capillary gas chromatography: analysis of *Mycobacterium gordonae* and *Mycobacterium scrofulaceum*. *Journal of General Microbiology* **129**, 401–405.

ANDERSEN, O., JANTZEN, E., CLOSS, O., HARBOE, M., SAXEGAARD, F. & FODSTAD, F. (1982). Fatty acid and polar lipid analysis as tools in the identification of *Mycobacterium leprae* and some related slow-growing mycobacterial species. *Annales de Microbiologie* **133B**, 29–37.

ASSELINEAU, C., CLAVEL, S., CLÉMENT, F., DAFFÉ, M., DAVID, H., LANÉELLE, M. A. & PROMÉ, J. C. (1981). Constituants lipidiques de *Mycobacterium leprae* isolé de tatou infecté expérimentalement. *Annales de Microbiologie* **132A**, 19–30.

CONVIT, J., ARANZAZU, N., ULRICH, M., PINARDI, M. E., REYES, O. & ALVARADO, J. (1982). Immunotherapy with a mixture of *Mycobacterium leprae* and BCG in different forms of leprosy and in Mitsuda-negative contacts. *International Journal of Leprosy* **50**, 415–424.

DAFFÉ, M., LANÉELLE, M. A., PROMÉ, D. & ASSELINEAU, C. (1981). Étude des lipides de *Mycobacterium gordonae* comparativement à ceux de *M. leprae* et de quelques mycobactéries scotochromogènes. *Annales de Microbiologie* **132B**, 3–12.

DAFFÉ, M., LANÉELLE, M. A., ASSELINEAU, C., LÉVY-FRÉBAULT, V. & DAVID, H. (1983). Intérêt taxonomique des acides gras des mycobactéries: proposition d'une méthode d'analyse. *Annales de Microbiologie* **134B**, 241–256.

DOBSON, G., MINNIKIN, D. E., MINNIKIN, S. M., PARLETT, J. H., GOODFELLOW, M., RIDELL, M. & MAGNUSSON, M. (1985). Systematic analysis of complex mycobacterial lipids. In *Chemical Methods in Bacterial Systematics*, pp. 237–265. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

DRAPER, P. (1976). Cell walls of *Mycobacterium leprae*. *International Journal of Leprosy* **44**, 95–98.

DRAPER, P., DOBSON, G., MINNIKIN, D. E. & MINNIKIN, S. M. (1982). The mycolic acids of *Mycobacterium leprae* harvested from experimentally infected nine-banded armadillos. *Annales de Microbiologie* **133B**, 39–47.

DRAPER, P., PAYNE, S. N., DOBSON, G. & MINNIKIN, D. E. (1983). Isolation of a characteristic phthiocerol dimycocerosate from *Mycobacterium leprae*. *Journal of General Microbiology* **129**, 859–863.

ETÉMADI, A. H. & CONVIT, J. (1974). Mycolic acids from 'noncultivable' mycobacteria. *Infection and Immunity* **10**, 235–239.

HUNTER, S. W. & BRENNAN, P. J. (1983). Further specific extracellular phenolic glycolipid antigens and a related diacylphthiocerol from *Mycobacterium leprae*. *Journal of Biological Chemistry* **258**, 7556–7562.

JULÁK, J., TUREČEK, F. & MIKOVÁ, Z. (1980). Identification of characteristic branched-chain fatty acids of *Mycobacterium kansasii* and *gordonae* by gas chromatography–mass spectrometry. *Journal of Chromatography* **190**, 183–187.

KUSAKA, T. & IZUMI, S. (1983). Gas chromatography of constitutive fatty acids in *Mycobacterium leprae*. *Microbiology and Immunology* **27**, 409–414.

KUSAKA, T., KOHSAKA, K., FUKUNISHI, Y. & AKIMORI, H. (1982). Isolation and identification of mycolic acids in *Mycobacterium leprae* and *Mycobacterium lepraemurium*. *International Journal of Leprosy* **49**, 406–416.

MINNIKIN, D. E., HUTCHINSON, I. G., CALDICOTT, A.

B. & GOODFELLOW, M. (1980). Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *Journal of Chromatography* **188**, 221–233.

MINNIKIN, D. E., MINNIKIN, S. M. & GOODFELLOW, M. (1982). The oxygenated mycolic acids of *Mycobacterium fortuitum*, *M. farcinogenes* and *M. senegalense*. *Biochimica et biophysica acta* **712**, 616–620.

MINNIKIN, D. E., DOBSON, G. & HUTCHINSON, I. G. (1983*a*). Characterization of phthiocerol dimycocerosates from *Mycobacterium tuberculosis*. *Biochimica et biophysica acta* **753**, 445–449.

MINNIKIN, D. E., MINNIKIN, S. M., DOBSON, G., GOODFELLOW, M., PORTAELS, F., VAN DEN BREEN, L. & SESARDIC, D. (1983*b*). Mycolic acid patterns of four vaccine strains of *Mycobacterium bovis* BCG. *Journal of General Microbiology* **129**, 889–891.

MINNIKIN, D. E., MINNIKIN, S. M., O'DONNELL, A. G. & GOODFELLOW, M. (1984*a*). Extraction of mycobacterial mycolic acids and other long-chain compounds by an alkaline methanolysis procedure. *Journal of Microbiological Methods* **2**, 243–249.

MINNIKIN, D. E., MINNIKIN, S. M., PARLETT, J. H., GOODFELLOW, M. & MAGNUSSON, M. (1984*b*). Mycolic acid patterns of some species of *Mycobacterium*. *Archives of Microbiology* **139**, 225–231.

MINNIKIN, D. E., PARLETT, J. H., MAGNUSSON, M., RIDELL, M. & LIND, A. (1984*c*). Mycolic acid patterns of representatives of *Mycobacterium bovis* BCG. *Journal of General Microbiology* **130**, 2733–2736.

MINNIKIN, D. E., DOBSON, G. & DRAPER, P. (1985). The free lipids of *Mycobacterium leprae* harvested from experimentally infected nine-banded armadillos. *Journal of General Microbiology* **131**, 2007–2011.

MIWA, T. K., MIKOLAJCZAK, K. L., EARLE, F. R. & WOLFF, I. A. (1960). Gas chromatographic characterization of fatty acids. *Analytical Chemistry* **32**, 1739–1742.

SHEPARD, C. C., DRAPER, P., REES, R. J. W. & LOWE, C. (1980). Effect of purification steps on the immunogenicity of *Mycobacterium leprae*. *British Journal of Experimental Pathology* **61**, 376–379.

SHEPARD, C. C., VAN LANDINGHAM, R. M., WALKER, L. L. & YE, S.-Z. (1983). Comparison of the immunogenicity of vaccines prepared from viable *Mycobacterium bovis* BCG, heat killed *Mycobacterium leprae*, and a mixture of the two for normal and *M. leprae*-tolerant mice. *Infection and Immunity* **40**, 1096–1103.

TISDALL, P. A., ROBERTS, G. D. & ANHALT, J. P. (1979). Identification of clinical isolates of mycobacteria with gas–liquid chromatography alone. *Journal of Clinical Microbiology* **10**, 506–514.

WORLD HEALTH ORGANIZATION (1980). UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. Report of the Fifth Meeting of the Scientific Working Group on the Immunology of Leprosy (IMMLEP). TDR/IMMLEP-SWG(5)/80.3, Annex 4, p. 23. Geneva: World Health Organization.

YOUNG, D. B. (1980). Identification of *Mycobacterium leprae*: use of wall-bound mycolic acids. *Journal of General Microbiology* **121**, 249–253.