Free Mycolic Acids as Criteria in the Classification of *Gordona* and the 'rhodochrous' Complex

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SUMMARY

The methyl esters of free mycolic acids from representative strains of *Gordona bronchialis*, *G. rubra*, *G. terrae* and *Nocardia kirovani* each gave, on mass spectroscopy, homologous series of anhydromycolic esters containing from one to four double bonds with the main components of the parent mycolic acids centred on 56, 58, 62 or 64 carbon atoms (total range from C₅₂ to C₆₈). The mycolic acids from the *Gordona* strains, with chain lengths centred around C₆₀, form a group intermediate in size between nocardomycolic acids (centred around C₅₀) and mycolic acids *sensu stricto* (centred around C₇₀ to C₉₀); these are different from those of the 'rhodochrous' complex which have anhydromycolates ranging from C₃₄ to C₅₀. *Gordonae* are thus more closely related in their mycolic acid composition to *Nocardia* than to *Mycobacterium* but can be distinguished from each of these genera.

INTRODUCTION

The genus *Gordona* (Tsukamura, 1971) was proposed for a group of slightly acid-fast bacteria isolated from sputa of patients with pulmonary disease and also from soil. The isolates, classified as *Gordona bronchialis*, *G. rubra* and *G. terrae*, were readily differentiated from nocardiae and mycobacteria but were difficult to distinguish from rhodochrous strains. The data accumulated so far indicate that rhodochrous strains form a recognizable, but heterogeneous, taxon equivalent in rank to the genera *Nocardia* and *Mycobacterium* (Cross & Goodfellow, 1973; Alshamaony, Goodfellow & Minnikin, 1976; Goodfellow *et al.* 1974). The relationship between the genus *Gordona* and the 'rhodochrous' complex, however, remains unsettled though Tsukamura (1973, 1974) has proposed that *gordonae* and rhodochrous strains be classified in a single genus with three new species, *Gordona rhodochroa*, *G. rosea* and *G. rubropertincta* (syn. *G. rubra*), erected for strains previously classified in the 'rhodochrous' complex.

Nocardiae, mycobacteria and true corynebacteria can be distinguished from one another by analysis of their respective mycolic acid moieties (Lechevalier, Horan & Lechevalier, 1971; Lechevalier, Lechevalier & Horan, 1973). The mycolic acids of the rhodochrous cluster (Goodfellow, 1971) have significantly lower molecular weights than those from true nocardiae (Minnikin, Patel & Goodfellow, 1974; Alshamaony *et al.* 1976). Similar lipid

Table 1. *Designation and sources* of test strains*

N654	<i>Gordona bronchialis</i> , NCTC10667; M. Tsukamura, 3410; H. Kondo. ATCC25592. Sputum, pulmonary lesion. Type strain
N661	<i>G. bronchialis</i> , strain T2, M. Tsukamura, 3411. Sputum, pulmonary lesion
N655	<i>G. rubra</i> , NCTC10668; M. Tsukamura, 3605. ATCC25593. Soil
N657	<i>G. rubra</i> , strain T3; M. Tsukamura, 3605. Soil
N658	<i>G. rubra</i> , strain T4; M. Tsukamura, 3606. Soil
N656	<i>G. terrae</i> , NCTC10669; M. Tsukamura, 3612. ATCC25594. Soil
N659	<i>G. terrae</i> , strain T5; M. Tsukamura, 3612. Soil
N662	<i>G. terrae</i> , strain T6; M. Tsukamura, 3613. Soil
N647	<i>Nocardia kirovani</i> , G. Michel, Universite Claude Bernard, Villeurbanne, France, IM1374

* ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.; NCTC, National Collection of Type Cultures, London; N, Laboratory number.

analyses of *Gordona* strains might clarify their relationship with rhodochrous strains, nocardiae and mycobacteria. This report presents results of the mass spectral analysis of methyl esters of free mycolic acids from representative strains of *Gordona bronchialis*, *G. rubra* and *G. terrae*, and from a strain of *Nocardia kirovani*.

METHODS

Strains and growth conditions. Details of the cultures examined are given in Table 1. All the strains were maintained on yeast extract agar slopes at room temperature.

The test strains were grown in shake culture at 37 °C for 3 to 5 days in modified Sauton's medium (Mordarska, Mordarski & Goodfellow, 1972) amended with vitamin B₁ (50 mg/l). Cultures were checked for purity at maximum growth, killed by shaking with formalin (1 %, v/v), separated by centrifuging, washed with distilled water and freeze-dried.

Preparation of methyl esters of free mycolic acids and their mass spectroscopy. The free mycolic acids were isolated, purified, esterified and then examined by mass spectroscopy as described by Alshamaony *et al.* (1976). Partial mass spectra of the methyl esters of the free mycolic acids of all the strains listed in Table 1 have been deposited with the British Lending Library as Supplementary Publication No. SUP 28004 (9 pages); for details of how to obtain copies, see Alshamaony *et al.* (1976); price 25p per copy (65p to Europe, 75p elsewhere).

RESULTS

Methyl esters of mycolic acids from *Gordona* strains and *N. kirovani* fragment on mass spectrometry in the same way as those of nocardiae (Maurice, Vacheron & Michel, 1971); the process is described in the accompanying paper (Alshamaony *et al.* 1976). The main peaks in the mass spectra of the methyl esters of the free mycolic acids from these strains correspond to fragments associated with the long alkyl branch in the 2-position (Table 2), anhydromycolates (Table 3), and aldehydes which, as noted in the accompanying paper, are variable in intensity.

DISCUSSION

Numerical taxonomic data indicate that *Gordona* has a closer affinity to the genus *Nocardia* than to the genus *Mycobacterium*, but shows an even greater similarity to the 'rhodochrous' complex from which it cannot be readily separated. However, using *in vitro* antimicrobial sensitivity tests, Goodfellow & Orchard (1974) distinguished between gordonae (which were resistant to rifampicin) and rhodochrous strains (which were all inhibited by this antibiotic).

Table 2. Peaks in the mass spectra of methyl mycolates of *gordoniae* and *N. kirovani* corresponding to fragments derived from the 2-branched 3-hydroxyester unit

The main component of each series is denoted by + + +, any component greater than 50 % of the main peak by + + and all other significant components by +.

Mass spectral peaks corresponding to cleavage*		a m/e	270	298
		b m/e	290	327
Branch in 2-position*			C ₁₄ H ₂₉	C ₁₆ H ₃₃
Straight-chain ester*			C ₁₆	C ₁₈
Strain				
<i>G. bronchialis</i>	N654		+ +	+ + +
	N661		+	+ + +
<i>G. rubra</i>	N655		+ +	+ + +
	N657		+ +	+ + +
	N658		+ +	+ + +
<i>G. terrae</i>	N656		+ +	+ + +
	N659		+ +	+ + +
	N662		+ +	+ + +
<i>N. kirovani</i>	N647		+ + +	+

* Explanation of assignment of peaks is given in the accompanying paper (Alshamaony *et al.* 1976).

The anhydromycolic esters of *Gordona* and *Nocardia kirovani* strains consist of homologous series containing from one to four double bonds with the main components of the parent mycolic acids centring on C₅₆, C₅₈, C₆₂ or C₆₄, with a total range from C₅₂ to C₆₆ (Table 3). Although the range of chain lengths, and the major components, of the mycolic esters show a degree of variation there is a reasonably consistent pattern within each species. The two strains of *Gordona bronchialis* produced mycolates from C₅₄ to C₆₆ (major components C₆₂ to C₆₄). *Nocardia kirovani* contained free mycolic acids having a range (C₅₉ to C₆₅) similar to that (C₅₈ to C₆₆) recorded by Maurice *et al.* (1971). A second group was formed by the mycolates from the *G. rubra* and *G. terrae* strains, ranging from C₅₂ to C₆₂. This suggests that the taxa examined are characterized by a particular type of mycolic acid with a chain length centred around C₆₀. The apparent affinity between *N. kirovani* and the *gordoniae* should be examined in more detail.

The free molecular weights of the mycolic acids from *G. bronchialis*, *G. rubra* and *G. terrae* are significantly higher than those from nocardiae and rhodochrous strains, but in general are very much lower than those from mycobacteria. The free mycolic acids from the *gordoniae* range from C₅₂ to C₆₆, thereby forming molecular species which can be distinguished from nocardomycolic acids (C₄₆ to C₅₈), mycolic acids *sensu stricto* (C₇₀ to C₉₀) and those which characterize many rhodochrous strains (C₃₄ to C₅₀) (Alshamaony *et al.* 1976). These results support the view that the *gordoniae* are more closely related to *Nocardia* than to *Mycobacterium* but can be distinguished from each of these genera (Tsukamura, 1971). It also seems likely that structural analysis of methyl mycolate moieties may provide an additional way of distinguishing between *gordoniae* and many rhodochrous strains.

A 14 or 16 carbon ester is the major product detected by mass spectrometry of mycolates from nocardiae and rhodochrous strains (Alshamaony *et al.* 1976), whereas the major esters released on pyrolysis of methyl mycolates of mycobacteria are usually in the range C₂₂ to C₂₆ (Lechevalier *et al.* 1971, 1973). However, the major product on mass spectrometry of *Gordona* mycolates was an 18 carbon ester, which also suggests that *gordoniae* are intermediate between nocardiae and mycobacteria.

Table 3. *Peaks in the mass spectra of methyl mycolates of gordonae and N. kirovani corresponding to anhydromycolates*

The main component of each series is denoted by + + +, any component greater than 50 % of the main peak by + + and all other significant components by +.

No. of carbons in parent mycolic acid	<i>m/e</i>	No. of double bonds	<i>G. bronchialis</i>		<i>G. rubra</i>			<i>G. terrae</i>			<i>N. kirovani</i> N647
			N654	N661	N655	N657	N658	N656	N659	N662	
C ₅₂	764	4	.	.	.	+	+	+	.	+	.
	766	3	.	.	+	+	+	+	+	+	.
	768	2	.	.	++	+	+	+	+	+	.
	770	1	.	.	+	.	.	+	+	.	.
C ₅₃	780	3	.	.	.	.	.	.	+	+	.
	782	2	.	.	.	.	.	.	+	.	.
	784	1	.	.	.	.	.	.	+	.	.
C ₅₄	792	4	+	.	+	++	++	++	.	+	.
	794	3	+	.	++	++	++	++	+	+	.
	796	2	+	.	++	.	.	+	+	+	.
	798	1	+	.	++	.	.	+	+	.	.
C ₅₅	806	4	.	.	.	.	+	+	.	.	.
	808	3	+	.	+	+	+	+	+	+	.
	810	2	+	.	+	+	+	+	+	.	.
	812	1	.	.	+	.	.	.	+	.	.
C ₅₆	820	4	+	.	++	++	++	+	.	+	.
	822	3	++	.	++	++	++	++	.	++	.
	824	2	++	.	++	.	+	++	+	.	.
	826	1	.	.	++	.	.	.	+++	.	.
C ₅₇	834	4	.	.	+	+	+	+	.	+	.
	836	3	+	.	++	++	++	++	++	++	.
	838	2	+	.	++	+	+	+	+	+	.
	840	1	+	.	+	+	+	.	+	+	.
C ₅₈	848	4	+	+	+	+	+	+	.	+	.
	850	3	++	+	++	+++	+++	+++	+	+++	.
	852	2	++	.	+++	++	++	++	+	+	.
	854	1	.	.	++	.	.	.	++	.	.
C ₅₉	862	4	.	.	+	+	+	+	.	+	+
	864	3	+	+	++	++	++	+	.	+	+
	866	2	+	.	+	+	+	+	+	+	.
	868	1	+	.	+	+	+	+	+	+	.
C ₆₀	876	4	+	+	+	+	+	+	.	+	+
	878	3	++	+	++	++	++	++	.	+	+
	880	2	+	.	++	++	++	.	+	.	.
	882	1	.	.	.	.	.	.	+	.	.
C ₆₁	890	4	+	+	+	+	+	+	.	+	++
	892	3	+	+	+	+	+	.	.	+	.
	894	2	+	+	+	+	+	.	+	+	.
	896	1	.	.	+	+	+	.	.	+	.
C ₆₂	904	4	++	++	+	+	+	+	.	.	+++
	906	3	+++	++	+	+	+	+	.	.	++
	908	2	.	.	+	+	+	.	.	.	.
C ₆₃	918	4	+	++	.	.	.	.	.	.	++
	920	3	+	+	.	.	.	.	.	.	+
	922	2	+	+	.	.	.	.	.	.	+
	924	1	+	.	.	.	.	.	.	.	.
C ₆₄	932	4	++	+++	.	.	.	+	.	.	+
	934	3	+	+	.	.	.	.	.	.	.
C ₆₅	946	4	+	+	.	.	.	.	.	.	+
	948	3	+	+	.	.	.	.	.	.	+
	950	2	+	+	.	.	.	.	.	.	+
C ₆₆	960	4	.	+	.	.	.	.	.	.	.
	962	3	.	+	.	.	.	.	.	.	.

Analysis of the mycolic acid moieties separates strains of *Gordona* (Tsukamura, 1971) from those in the 'rhodochrous' complex (Goodfellow, 1971) but the sharp distinction between these taxa is probably more apparent than real. Thus, three rhodochrous strains (ATCC4273, ATCC13808 and N31), classified as *Gordona rhodochroa* (Tsukamura, 1973), produce anhydro-mycolic esters which consist of homologous series containing zero to two double bonds and mycolates ranging from C₃₈ to C₄₈ (major component C₄₄) (Alshamaony *et al.* 1976). Although it is difficult to compare phena from different numerical taxonomic studies it is apparent that *G. rhodochroa* (Tsukamura, 1973, 1974) is substantially the same taxon as *Nocardia rubra* (Bradley, 1971), and the rhodochrous subclusters 14C (Goodfellow, 1971) and 1a (Goodfellow *et al.* 1974). Again, further centres of variation have been discovered in the 'rhodochrous' complex (Goodfellow *et al.* 1974) and there is some evidence that one of the new phena contains strains with mycolic acids similar to those of true nocardiae (Alshamaony *et al.* 1976). It seems probable, therefore, that mycolic acids with different chain lengths will be characteristic of different *Gordona* and rhodochrous subclusters. If the 'rhodochrous' complex and the proposed genus *Gordona* were merged to form a single genus, the structure of the mycolic acids might be of value in differentiation at the subgeneric level.

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